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OBSERVATIONS ON THE EFFECT OF ENVIRONMENTAL CONDITIONS ON THE STRUCTURE OF THE LATERAL ROOTS IN SUGAR BEET [1]

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INTRODUCTION

Plants of sugar beet (*Beta vulgaris* L.) at State College, N. Mex., which were being grown for seed production by the overwintering-in-the-field method in the fall of 1935 and spring of 1936, afforded an opportunity for the study of growth and development under a variety of conditions. In the experimental plantings [2] the influence of previous cropping practices upon seed yields was being determined by a series of replicated plots. In this series of plots during the spring and summer of 1935 such crops as cantaloups (*Cucumis melo* L.), cowpeas (*Vigna sinensis* (Torner) Savi), *Sesbania* spp., corn (*Zea mays* L.), and Sudan grass (*Sorghum vulgare* var. *sudanense* (Piper) Hitchc.) had been grown, and the effects of these crops on the subsequent sugar-beet seed crop, in contrast to fallowing the soil, were under test. In the fall of 1935 noticeable differences in the top growth and apparent vigor of the sugar-beet plants were evident, the fallow plots showing greatest growth; the preceding crops in their effects could be ranked in the order given, *Sesbania*, corn, and Sudan grass being definitely retarding in their influence on fall growth. The inequality found in the fall was apparent even after growth started in 1936. However, about the middle of March the different plots began to take on a more uniform appearance. With the advent of seedstalk formation, plots that had made a relatively poor showing in the fall, such as those previously cropped to corn, could hardly be distinguished from the better looking plots of the series.

The variations in soil type, field contour, insect infestation, and previous handling made it possible to secure from the experimental plots a number of collections of sugar-beet roots representing a range of exposures to diverse environmental conditions. Attempt was made by study of lateral root structure to link the deviations from normal development with the environmental conditions which obtained in the soil surrounding the roots selected. The study was limited to the lateral root structures and is presented as a preliminary approach in a field of research about which there exists little information.

MATERIAL AND METHODS

The material for study was obtained from the overwintered sugar-beet plants, after active growth had been resumed in the spring. The

[1] Received for publication Oct. 29, 1936; issued August 1937.

[2] Cooperative experiments on sugar-beet seed production of the New Mexico Agricultural Experiment Station and the Division of Sugar Plant Investigations, Bureau of Plant Industry, U. S. Department of Agriculture.

sugar-beet seed had been planted in early September; development of the seedlings was rapid during the fall and early winter. Growth continued even during the brief winter months and was actively resumed early in March. The plants to be examined were dug with a large clump of the adobe soil adhering and taken to the laboratory. The plants with the adhering soil were soaked in a large tub filled with water. The loosened soil was then carefully removed, after which each taproot with the laterals was placed in a shallow dish of water. The rootlets were cut off close to the taproot and tied together in bundles of about 100 each. The bundles were then cut to a length of three-quarters of an inch, fixed, embedded, and cut as single units. This arrangement made possible the handling of a large number of rootlets and facilitated comparative study under the microscope. In one or two cases where the stand was sparse only two taproots were used as a source of lateral rootlets, but in most cases five or six taproots furnished the lateral rootlets used in this study. The material was fixed in Karpechenko solution and stained with Delafield haematoxylin.

DISTRIBUTION, ORIGIN, AND STRUCTURE OF LATERAL ROOTLETS

The lateral rootlets of the sugar beet are filiform and generally are spread horizontally, remaining confined mostly to the topsoil to the depth of a deep furrow. According to Andrews,[3] the lateral roots permeate every cubic centimeter of topsoil to a depth of 8 to 14 inches and extend laterally 2 feet or more. The adobe nature of the soil made it difficult to trace the individual rootlets for more than 15 inches, but the observations by Andrews cited above and those of European investigators[4] show conclusively that the horizontal spread of the laterals extends some distance beyond the confines of the individual rows.

The lateral roots are whitish and, for any given taproot, of approximately equal diameter, save for a few scattered individuals. They occur in two more or less distinct rows along the flattened side of the taproot. As is usual for lateral rootlets, those of the beet have their origin in the pericycle (pl. 1, *A*); but, as the taproot increases in thickness, laterals that are later formed arise from the more peripheral rings of growth. The rootlets are covered for a considerable distance with rather long root hairs that remain alive for a long time.

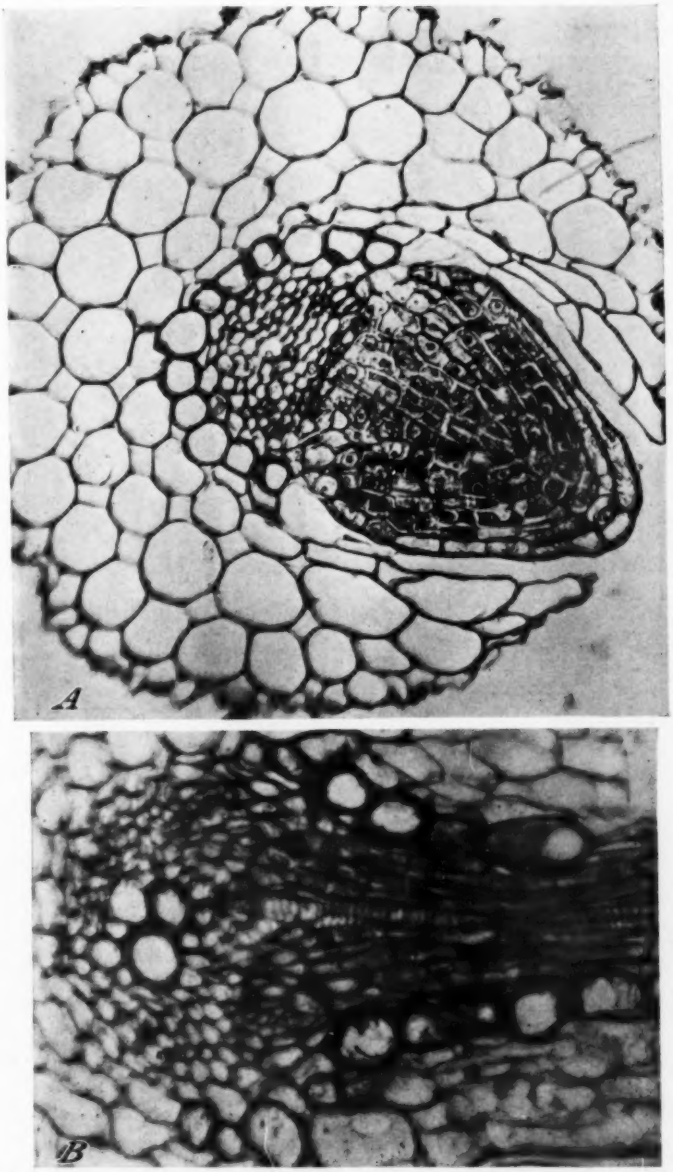
The anatomical structure of a lateral rootlet is simple; each rootlet consists of a broad though few-celled cortex surrounding a miniature stele with the usual type of tissue arrangement (fig. 1). This structure differs from that of the taproot in its protoxylem plate, in the absence of secondary growth, and in the lack of periderm.

CENTRAL CYLINDER WITH ITS ENDODERMIS

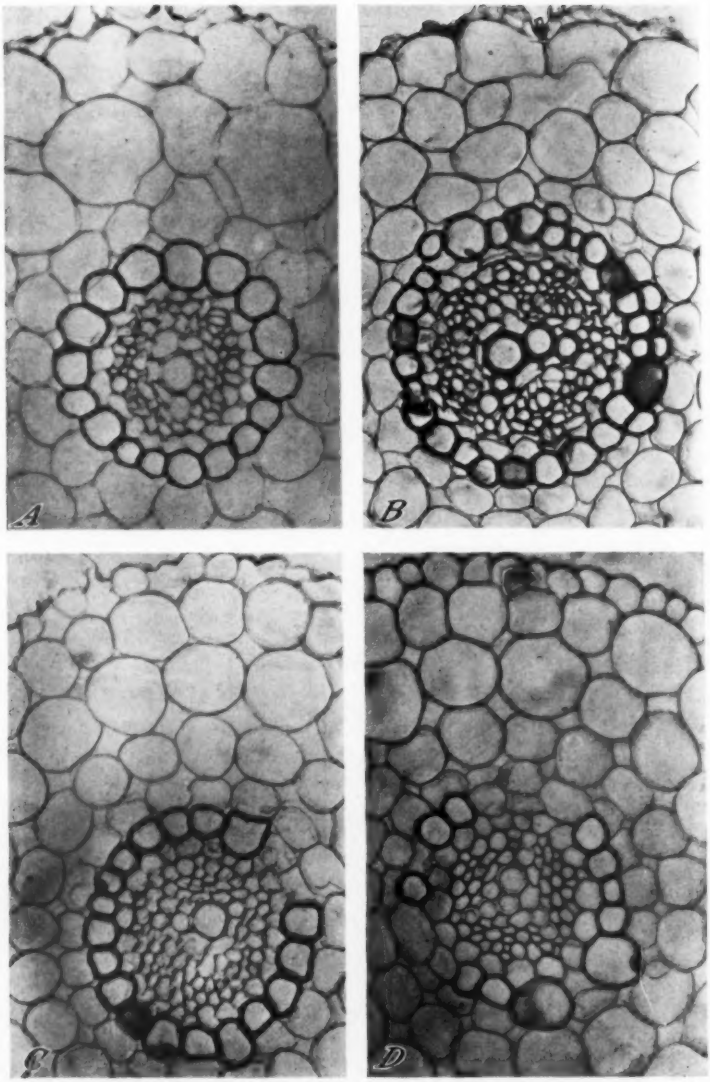
The central cylinder is made up of the protoxylem plate with alternating phloem groups, a single-layered pericycle, and a band of parenchyma between xylem and phloem, in which a few tangential divisions, the initials of a rudimentary cambium, are discernible. Similar tangential divisions may be observed in the pericycle, and

[3] ANDREWS, L. H. THE RELATION OF THE SUGAR BEET ROOT SYSTEM TO INCREASED YIELD. Through the Leaves 15: 17–20, illus. 1927.

[4] VRIES, H. DE. BEITRÄGE ZUR SPECIELLEN PHYSIOLOGIE LANDWIRTSCHAFTLICHER CULTURPFLANZEN. Landw. Jahrb. 8: [417]–498, illus. 1879.



A, Cross section of lateral rootlet with emerging tertiary root. The thickened endodermal cells form an unbroken semicircle. *B*, Thick-walled endodermal cells of tertiary root continuous with those of parent root which has a broken type of endodermis. Both × 450.



A, Transverse section of lateral rootlet with unbroken type of endodermis. *B*, Unbroken thick-walled endodermis two cells wide in places. *C*, Partially broken type of thick-walled endodermis. *D*, Broken type of thick-walled endodermis. Note that the thin-walled cells lie opposite the protoxylem points. All × 450.

although a complete phellogen may occasionally be formed, a periderm is never developed.

The protoxylem plate of the taproot is diarch. In the lateral rootlets such plates are not uncommon, but the bulk of the laterals is triarch, and in larger rootlets, though not limited to them, tetrarch plates are often found. The protoxylem of the plates of older rootlets

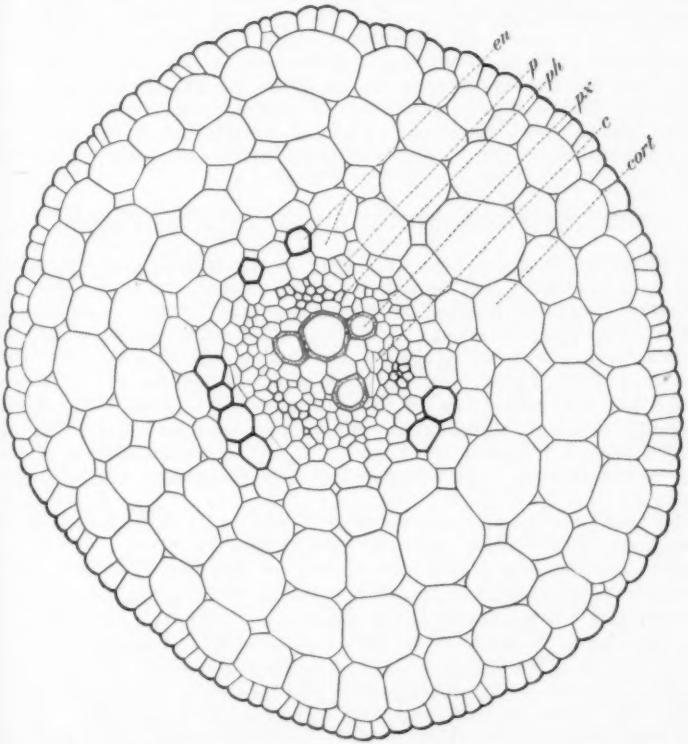


FIGURE 1.—Cross section of lateral rootlet of sugar beet: *en*, Endodermis composed of thick- and thin-walled cells; *p*, pericycle (some of the cells have divided tangentially to form a partial phellogen); *ph*, phloem; *px*, protoxylem; *c*, cambium; *cort*, cortex. × 340.

is augmented by metaxylem elements that sometimes give the central part of the rootlet the appearance of a solid xylem core.

The endodermis is composed of a single layer of axially elongated cells which form a complete jacket around the central stele. The cells have the characteristic Casparian strips and in addition develop secondary thickenings which are of cellulose and stain intensely with Delafield haematoxylin. These secondary thickenings may be present in all endodermal cells (pl. 2, *A*), or they may be absent from certain sectors (pl. 2, *C*, *D*); sometimes they are altogether wanting. Differences in the patterns formed by the thickened cells result in three types of endodermis, as follows: (1) The "unbroken" type, designated in the tables as α, where the thickened cells form axially

uninterrupted rows; (2) the "slightly broken" type, designated as β, where there is an occasional interpolation of an unthickened member; and (3) the "broken" type, designated as γ, where there is a substitution of additional thin-walled cells. In the latter type the thin-walled cells usually lie opposite the protoxylem points (fig. 1 and pl. 2, *D*), but this relation does not always hold true. In the broken type of endodermis there is often a great difference in the sizes of the endodermal cells (pl. 2, *D*), a difference that is the more striking the greater the number of thin-walled cells. The ring of thickened endodermal cells is sometimes augmented by the development of secondary thickenings in adjacent cortical cells (pl. 2, *B*). At times secondary thickenings may even be found in certain pericyclic cells. Where branch roots depart from a lateral, a continuity of the thickened endodermal cells of the parent and branch root is usually maintained (pl. 1, *B*).

EFFECT OF ENVIRONMENT ON LATERAL ROOT SYSTEM

The influence of the preceding crop in the rotation on the lateral root system of the sugar beet was studied on material from plots that had previously been planted to corn, Sudan grass, *Sesbania*, or cowpea, or that had been left fallow. For data on the influence of unfavorable environment, plants were selected from waterlogged spots, poor sandy corners, and from areas in the plots in which the plants showed heavy infestation with plant lice on the young leaves. Most of the plants where growth conditions were unfavorable showed a yellowish discolored foliage and poor top growth. Samples were taken in March after resumption of active vegetative growth and again just before bolting.

Macroscopically, the root systems of the beets from the different plots were much alike, save for individual variation. When top growth was compared, one was struck by the fact that the plants from the rather poor-looking plots, such as those previously cropped to corn, had a root system equal and at times superior to those from the vigorous cowpea or fallow plots. Since the beets from the corn plot at time of bolting equaled those from the other field plots in vigor, it became quite evident that the well-developed root system of the beets from the corn plot was in a large measure responsible for their rapid subsequent development, notwithstanding their poor top growth showing during fall and early spring.

However, the factors that influenced grass top and root development had no appreciable effect on the inner root structure, as may be seen from a study of table 1. Taking as a criterion the large healthy plant with big taproot, no. 4 of table 1, the diameter of the laterals averaged a little more than 300μ, while the ratio of diameter of rootlet to diameter of the stele inclusive of the endodermis was approximately 2.51:1. The rootlets from the plot in which corn preceded sugar beets had a slightly larger stele, and the ratio of diameter of rootlet to diameter of stele was 2.70:1. This was a somewhat lower ratio than that observed in the rootlets from the cowpea or fallow plots.

The rootlets from plants growing under unfavorable environmental conditions gave less uniform data. However, the differences in structure were only quantitative in character, since pathological

disturbances, so often associated with abnormal environment, had no influence on the general make-up of the roots.

The majority of the rootlets, from whatever source, had three protoxylem points. Roots with predominantly diarch plates were observed on only one occasion (table 1, item 5). They came from a rather unfavorable location and often exhibited pathological disturbances in the phloem and adjacent tissues. The ratio of diameter of total root to diameter of stele, however, was approximately the same as that of roots from a normal environment.

TABLE 1.—*Measurements and microscopic data on the lateral roots of sugar-beet plants: Summary of data*

No.	Material: Description	Samples	Average diameter: Entire root		Average diameter: Stele		Ratio of total diameter of rootlet to that of stele	Predominant protoxylem points	Frequency of endodermis pattern			Remarks
												α β γ
		Number	μ	*S.E.*	μ	*S.E.*		*Number*	γ	β	α	
1	With corn as preceding crop.	15	333	±8.6	123	±4.6	2.70:1	3	6	6	3	
2	With cowpea as preceding crop.	13	343	±8.4	119	±5.0	2.88:1	3	5	6	2	
3	After fallow	10	355	±11.1	115	±5.1	3.08:1	3	5	2	3	
4	Large healthy plant with big tap root.	16	304	±12.8	121	±7.4	2.51:1	3	1	3	12	
5	Small yellow plant from sandy corner of field.	11	325	±19.3	128	±5.0	2.54:1	2	9	2	0	Many roots necrotic.
6	Plant with yellow leaves and infested with plant lice.	25	294	±11.5	123	±5.9	2.39:1	3	4	11	10	
7	Small plant from poor stand in low spot.	12	225	±13.0	83	±4.1	2.71:1	3	3	4	5	
8	Medium-sized yellow plant from waterlogged spot.	24	281	±31.2	90	±3.8	3.12:1	3	1	12	11	About 25 percent of the roots necrotic.

The three types of endodermis, as illustrated in plate 2, *A*, *C*, and *D*, and referred to in table 1 as endodermis patterns α, β, γ, were found in all root material, regardless of its source. Random observations on cross sections showed a prevalence of one or the other type, but this was seldom sufficiently definite to be significant. Roots from an apparently normal environment (from corn, cowpea, and fallow plots) showed the closed and broken types in fairly equal proportions, while the roots from plants growing in an unfavorable environment showed a preponderance of the broken type (table 1). An exception again is found in the roots from a plot previously noted as having mostly diarch protoxylem plates (table 1, item 5), in which the vascular cylinder was almost always jacketed with an endodermis of unbroken thick-walled cells.

PATHOLOGICAL DISTURBANCES

It would be strange, indeed, if among the large number of rootlets belonging to a plant only normal individuals should be found; and it is noteworthy that in the case of the lateral rootlets of sugar beets growing in what should be considered a normal environment the percentage of diseased individuals is so small. If diseased rootlets are present at all, the type of injury is usually slight and often mechanical in nature, being limited to a destruction of small sectors of cortex and

rarely reaching the vascular tissue. At times, the entire cortex is more or less collapsed while the vascular tissue is still normal and capable of conducting water and nutrients. True pathological disturbances in the vascular tissue are sometimes found in the protoxylem cells; these are seen filled with a gummy deposit, or may even contain micro-organisms.

In plants grown under seemingly disadvantageous conditions, especially when temporarily subjected to high water during the winter months, the number of diseased rootlets may be quite large and the pathological picture fundamental and severe. Diseased xylem cells become quite common and are often accompanied by pathological changes in the water-conducting cells or even by a diseased condition of the phloem (pl. 3, *A*). Phloem necrosis in its inception is limited to a few pericyclic cells, those elements that were shown to be so susceptible to virus invasion.[5]

The diseased pericyclic cells are usually few and do not hypertrophy like those affected by the curly top virus. At times they separate and a red gummy deposit is found lodged in the intercellular space formed between the cells of the endodermis and those of the pericycle. Usually, however, necrosis spreads from the pericycle to the phloem and the adjoining parenchyma cells, involving all phloem groups or being limited to one or two, depending on whether the root in question is diarch or has a larger number of protoxylem points. The necrotic lesions somewhat resemble those associated with the curly top disease, but, fundamentally, they rather belong to the type found in potato plants affected with the leaf roll disease.[6]

Necrosis in some of the rootlets has a stimulating effect on the phellogen of the still healthy sectors, causing the production of a partial multilayered periderm (pl. 3, *B*). A similar effect may also be noted on the normal cambium below the diseased phloem.

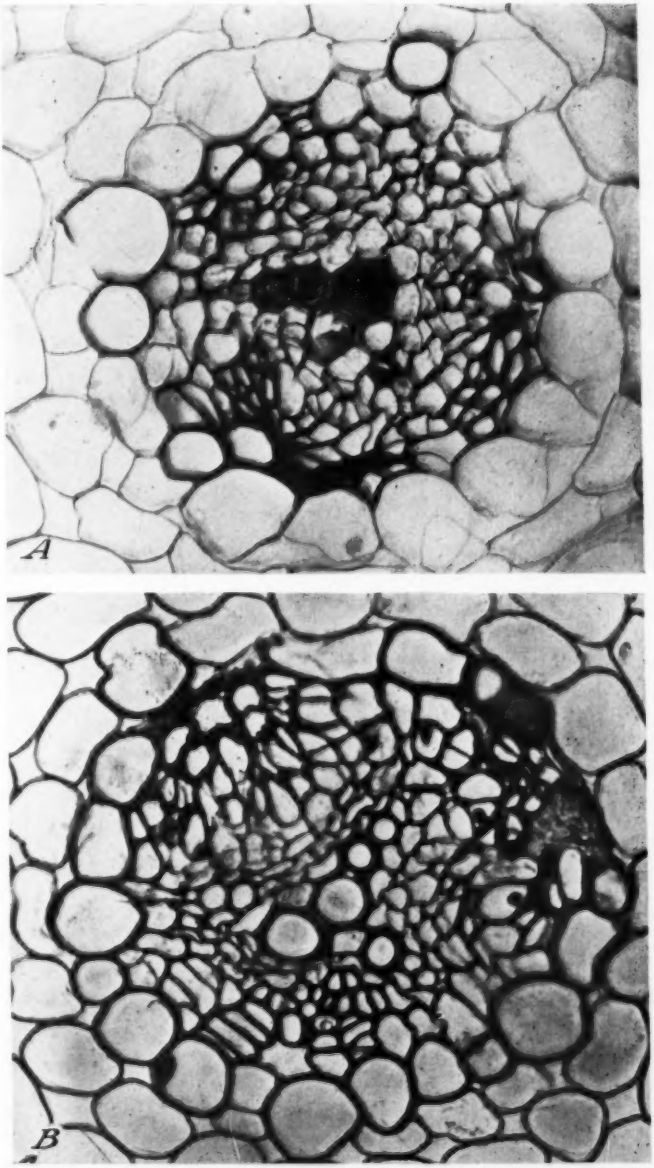
SUMMARY

Under normal conditions, the lateral rootlets of the sugar beet develop no secondary tissues and the protoxylem plates are predominantly triarch. The cells of the endodermis often develop secondary thickenings, forming an entire or partly broken thick-walled jacket around the vascular cylinder. If the thin-walled cells are few in number, they may well be likened to transfusion cells which are so common in a thick-walled endodermis.

The preceding crop in the rotation, while inhibiting early top growth in some plots (noticeably in beets after corn), apparently did not affect root development; e. g., the sugar beets from the "after-corn" plot, characterized by less top growth, showed a root system equal to that of the luxurious "after-cowpea" plot. No significant structural differences were noted in any of the material coming from an apparently normal environment; but distinct pathological changes, especially in the nature of phloem necrosis, were observed in plants grown under disadvantageous soil conditions.

[5] ARTSCHWAGER, E., and STARRETT, R. C. HISTOLOGICAL AND CYTOLOGICAL CHANGES IN BEETS AFFECTED WITH CURLY TOP. Jour. Agr. Research 53: 637–657, illus. 1936.

[6] ARTSCHWAGER, E. OCCURRENCE AND SIGNIFICANCE OF PHLOEM NECROSIS IN THE IRISH POTATO. Jour. Agr. Research 24: 237–245, illus. 1923.



A, Diseased lateral rootlet showing deposit in protoxylem cell and phloem necrosis. There is much variation in the size of the endodermal cells, most of which are thin-walled. *B*, Transverse section of diseased lateral rootlet. The rootlet is triarch with two of the phloem groups necrotic; above the third phloem group is a well-developed several-layered periderm. Both × 750.

EFFECT OF THE DWARF DISEASE ON THE ALFALFA PLANT[1]

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INTRODUCTION

In previous papers (*5*, *6*, *7*)[2] the writer has discussed the nature of the dwarf disease of alfalfa (*Medicago sativa* L.) and the effect of certain environmental factors on its spread and development. That the disease causes a considerable disturbance in the normal histology and physiology of the alfalfa plant is indicated by the dwarfing of the tops and the extensive discoloration in the roots. The results of certain histological and physiological studies made to obtain a better understanding of the nature of the effect of dwarf on the alfalfa plant form the basis of this paper.

HISTOLOGICAL METHODS

For the most part fresh material was used, although many roots and stems were fixed in formal-acetic alcohol, embedded in paraffin, sectioned, and stained. Some material was stored in fixative and sectioned with the sliding microtome without being embedded in paraffin.

The varieties studied were Hairy Peruvian and Chilean, and the plants were collected in the field at different times of the year.

Various stains were used, but Heidenhain's iron-alum haematoxylin and carbolfuchsin were the most helpful. Certain points, such as the yellowing of the vessels, could be studied best without staining.

THE DISEASE IN THE ROOT

When the bark is removed from diseased taproots in the winter and spring the yellow color observed during the growing season (*5*) usually is found to be more or less overgrown with a thin layer of new white wood (fig. 1). With the advent of cool weather in the autumn the disease becomes less active and gum[3] formation ceases, so that by late spring a layer of white wood 1 mm or more in thickness is present between the cambium and the yellow wood produced during the previous summer. The thickness of this new layer varies greatly in different roots and in different parts of the same root. A relatively thick layer of new wood may form on one side of the root, where the disease has not made such inroads, and little or none on the other side. A macroscopically perceptible layer of new wood may be present as early as December. The disease becomes active again the next

[1] Received for publication Oct. 3, 1936; issued August 1937. Cooperative investigations of the division of Forage Crops and Diseases, Bureau of Plant Industry, U. S. Department of Agriculture, and the division of Agronomy, California Agricultural Experiment Station.

[2] Reference is made by number (italic) to Literature Cited, p. 104.

[3] The term "gum" is used to designate the material plugging the vessels, regardless of its chemical composition.

spring and by midsummer has involved much of the new wood, and the plant slowly succumbs.

A microscopical study of cross sections of diseased roots shows clearly the parts affected. Little or no evidence of trouble of any kind appears in the bark, cambium, wood rays, or fibers of affected plants until the late stages of the disease, when there is usually some yellowing caused by a soluble stain. The parenchymatous cells contiguous to or near plugged vessels are sometimes affected, as is shown by the yellow color in their walls and their staining reaction. Sometimes there is a small amount of plasmolysis in these cells also. How-

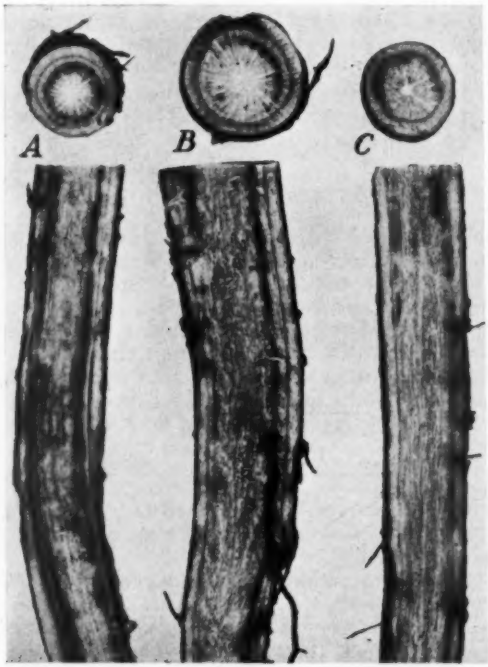

FIGURE 1.—Cross and longitudinal sections of three alfalfa roots affected with dwarf. The dark-colored streaks are regions where the vessels are plugged with gum. These roots show the early spring condition, when the discolored areas are covered by a layer of new growth. In cross section *A* the affected tissues form a complete dark-colored ring about 1 mm beneath the cambium. Cross section *B* shows a much wider band of diseased cells on the right and upper sides than elsewhere, and cross section *C* shows only a very small discolored region in the upper left-hand part. Compare with figure 3, which shows not only the unaffected region of winter growth covering the affected ducts beneath but also the layer of plugged cells nearer the cambium, developed during the current season. About natural size.

ever, in many cases, at least in the earlier stages of the disease, the parenchyma cells stain and appear quite normal. No X-bodies have ever been seen; the visible evidence of the disease is restricted for the most part to the vessels of the xylem.

Not all of the xylem is affected. The plugging at first may be limited to one or a few ducts in one or more bundles. Even in fairly advanced stages of disease certain bundles may be entirely unaffected while others are badly plugged (fig. 2). In fact, usually only a com-

paratively small proportion of the total number of ducts of a bundle are involved (figs. 2 and 3). The affected region in roots in which the disease is most active, as indicated by the formation of gum, lies just within the cambium; that is, in the part of the xylem that is functioning most actively in transporting water and nutrients. In advanced stages of the disease so many of the ducts in this region may be plugged that water cannot reach the tops in sufficient quantity to prevent wilting during the middle of the day in bright weather even when the soil moisture is very high.

FIGURE 2.—Cross section of alfalfa root having medium stage of dwarf. One bundle (*a*) has no plugged vessels, while the adjacent bundle on the left has several and the one on the right has two. Although unaffected bundles are often found, more frequently some ducts in all the bundles are more or less plugged, especially in the later stages of the disease. The affected region is several cells back from the cambium, a condition commonly seen during the winter and spring months. × 35.

The youngest vessels next to the cambium may be plugged but usually they are not except in the late stages of the disease. The plugging is most commonly in the second and third layer of ducts from the cambium. The width of the affected region increases as the disease develops. In the later stages the affected area usually occupies a zone not more than 2 or 3 mm in width, or, roughly, the outer third

of the distance from the center of the root to the cambium (fig. 3). Not much consistency is shown in the way the ducts are plugged. Sometimes the larger, sometimes the smaller, and sometimes all the ducts are involved. A single plugged duct or a group may be surrounded by ducts that apparently are unaffected. Likewise, one bundle may have a number of plugged ducts while an adjacent one

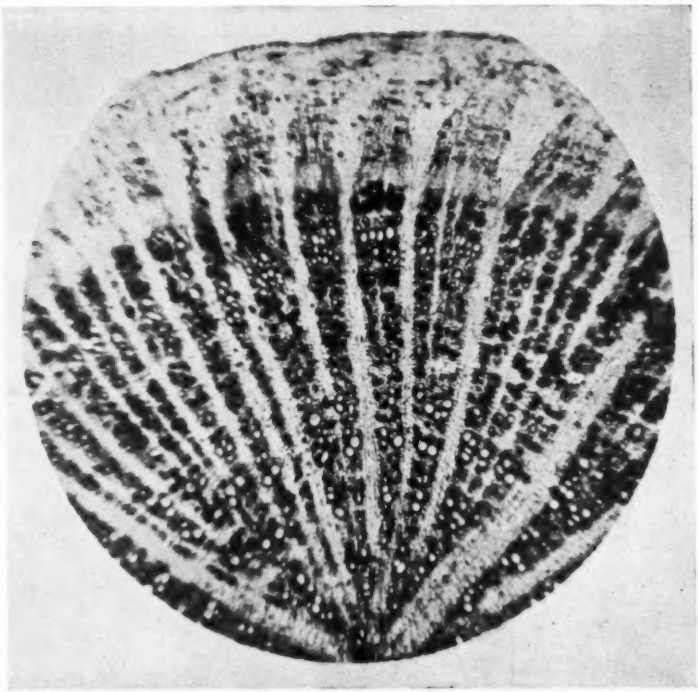

FIGURE 3.—Cross section of alfalfa root having late stage of dwarf. Three distinct bands can be distinguished in the outer half of the wood, two in which the vessels are plugged with gum, separated by a third which is almost unaffected. The band next to the cambium, which is the current season's growth, contains a large percentage of plugged ducts. Beneath this layer is the winter growth, in which the ducts are largely free of gum, although all or nearly all of the ducts in some bundles appear to be plugged. The previous season's growth, just beneath, also is badly affected. × 35.

has only a few or none at all (fig. 2). Many ducts lying end to end may be plugged, forming long strands of apparently continuous gum. The gum often shrinks and pulls away from the walls. Tyloses are sometimes present in the ducts.

THE DISEASE IN THE STEMS

It was pointed out in a previous paper (*5*) that the tops of affected plants show a gradual dwarfing which continues until the plant dies. It might be expected that the yellowing and plugging of the vascular system of the diseased roots would extend into the stems, but a study of many sections of stems has shown little if any stain and only a

small amount of plugging. Such little plugging as there is in the vessels of the green stems is found chiefly in the late stages and then only for a distance of an inch or two above the crown (fig. 4). The vessels of the stem that are plugged are usually though not always near the pith. The lack of extensive plugging in the stem tissue may be due, at least in part, to the fact that the disease progresses very

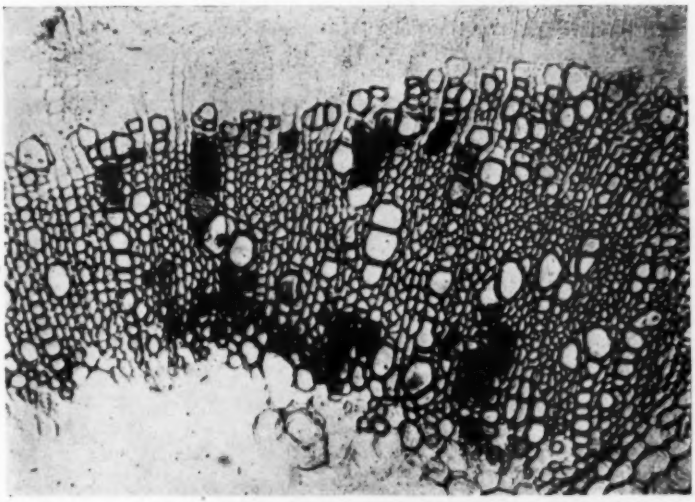

FIGURE 4.—Cross section of a portion of an alfalfa stem affected with dwarf. Several plugged ducts are evident, but plugging of the vessels in the stems is not extensive. Only the xylem is clearly shown. The pith is at the lower part of the picture. × 150.

slowly and that the stems are cut frequently (once every 28 to 34 days) during the growing season, when the disease is most active.

CHARACTER OF THE GUM

The gum may be homogeneous in character (figs. 5 and 6); it may have what appears to be a flocculent or granular precipitate; or it may contain numerous coccuslike or rod-shaped bacterialike bodies (fig. 7). The homogeneous and granular gum is comparable to that formed in many other plants. The bacterialike bodies are not present in the ducts of all affected plants; in fact, they have not been found at all in about 25 percent of the plants examined. Nevertheless they constitute one of the most reliable microscopical diagnostic characters of the disease.

From the beginning of these investigations the question whether or not these bodies are bacteria has presented itself. All efforts to prove by cultural methods that they are living entities have failed (7). Some bacteriologists and pathologists who have examined the material have unhesitatingly pronounced these bodies bacteria; others equally competent to judge have said that they were not bacteria. These bodies are limited to the ducts of the xylem. Pathogenic bacteria usually, if not always, sooner or later pass out of the vessels into the intercellular spaces of the parenchyma. The bodies in question are

embedded in a gum which holds them in clumps and keeps them from undergoing Brownian movement. When this gum is dissolved away by 50-percent chromic acid the bodies float free, showing that they are individuals and have a solubility slightly different from that of the gum. At times they appear yellow like the gum in which they are embedded.

The outer portion of these bacterialike bodies differs from the interior as seen both in stained and unstained preparations. The outer portion takes the stain while the inner remains largely or entirely unstained. This has been interpreted by some authorities as indicat-

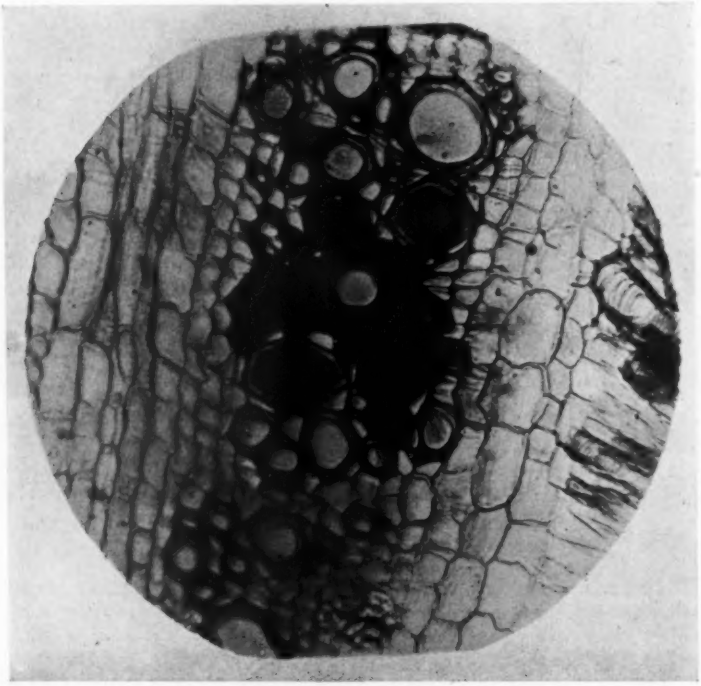

FIGURE 5.—Cross section of alfalfa root affected with dwarf showing group of plugged vessels in which some of the walls have been so changed or impregnated with gum that they have practically lost their identity Adjacent wood parenchyma cells have been affected to a limited extent. × 190.

ing the presence of a spore (figs. 7 and 8). In certain stages this outer portion takes a red color when treated with phloroglucin and hydrochloric acid, just as does the gum, indicating its similarity to wound gum. Neither these bodies nor the gum takes on this color in the early hyaline stage. So far as ascertained, no bacteria give this reaction for wound gum. Of course, it is possible that the outer portion of these bodies is impregnated with gum and so gives the characteristic gum reaction to the phloroglucin-hydrochloric acid treatment. These bodies are gram-negative and do not take many of the stains that most bacteria do, but rather stain like the gum

near or surrounding them. This fact suggests that these bodies are of a material similar to gum but that they differ from it slightly, at least in their solubility in chromic acid.

In the early stage these bodies are embedded in a hyaline material more or less creamy or semifluid in consistency. This was determined by stirring the material in a single duct under the microscope by means of a capillary glass needle manipulated by an apparatus similar to that described by Roberts (*4*). This material very soon hardens so that it can be moved about in a cell as a unit and the needle cannot penetrate it. Later the contents of the vessel turn yellow and give the wound-gum reaction as previously described. In the semifluid

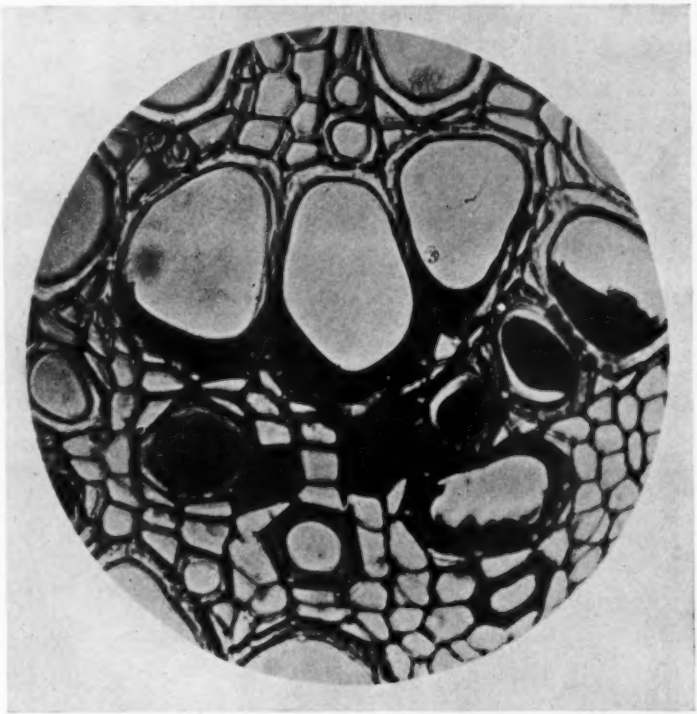

FIGURE 6.—Cross section of alfalfa root affected with dwarf, showing some ducts completely filled and others only partly filled with gum. For the most part the surrounding cells show no signs of disease, although there is a fairly profuse gumming just below the center one of the three large vessels. The upper parts of the walls of these three cells appear to be unaffected even though considerable gum has been formed lower down. × 370.

state the contents of the ducts are more or less readily washed away in the staining process. A few of the bodies were sometimes found isolated, lying along the wall of the duct and apparently free of gum. These often gave the red color when treated with phloroglucin and hydrochloric acid, which suggests that this reaction is due to the nature of the bodies themselves and not to the gum in which they are embedded. These bodies give a negative reaction for tannins, chitin,

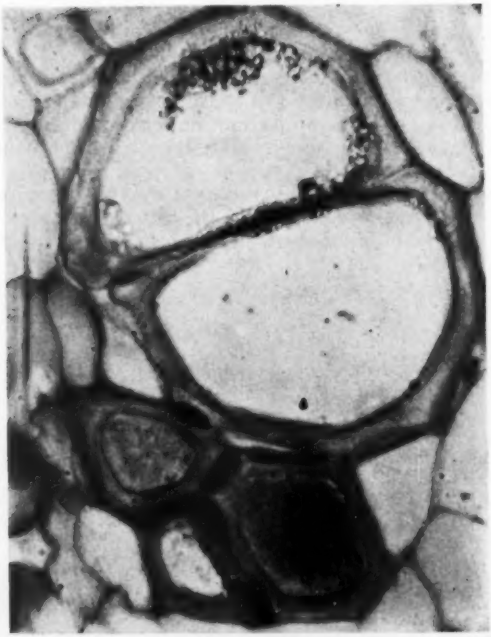

FIGURE 7.—Cross section of alfalfa root stained with carbolfuchsin, showing bacterialike bodies. These may be so numerous that they entirely plug the lumen of the vessel. × 1,100.

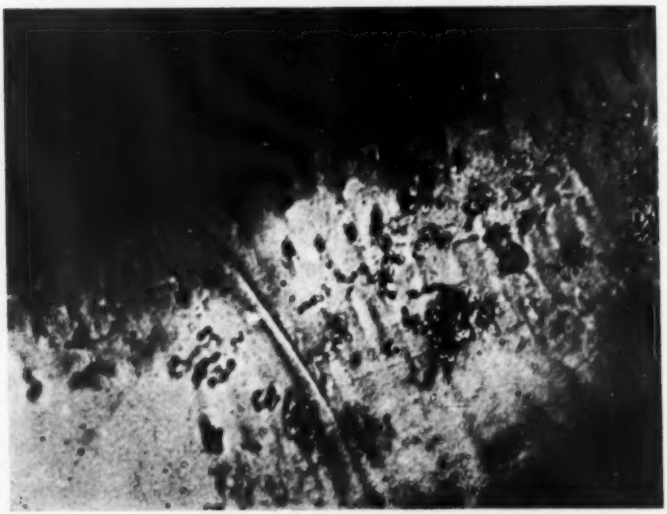

FIGURE 8.—Longitudinal section of a vessel in an alfalfa root stained with carbolfuchsin, showing bacteria like bodies scattered about. × 1,200.

protein, and fats and do not react to polarized light as crystals do. Knowledge of their exact chemical nature will have to await further study.

ORIGIN OF THE GUM

To determine with certainty the source of the gum plugging the vessels of alfalfa plants affected with dwarf is difficult. In the early stages of the disease certain portions of the walls become impregnated with gum, which eventually collects along their inner margins (figs. 9 and 10). Later the ducts may be partly or completely filled with

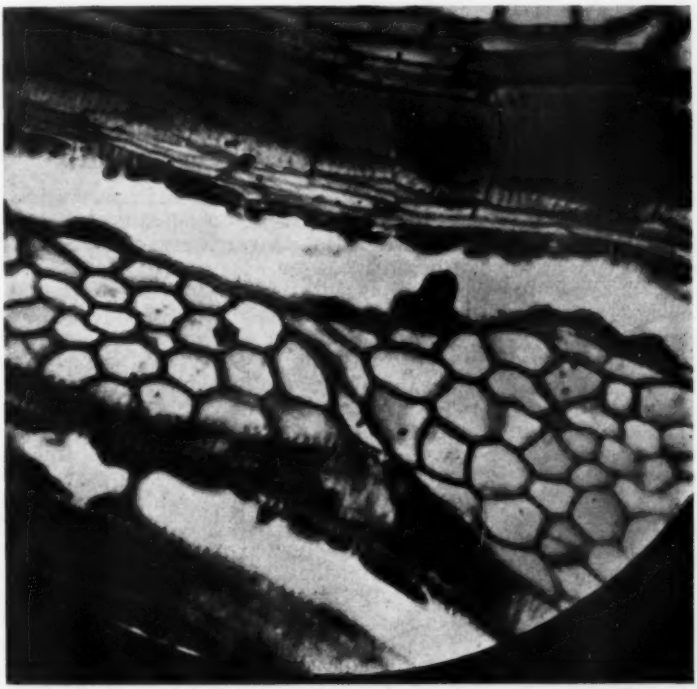

FIGURE 9.—Tangential longitudinal section of alfalfa root showing two vessels each with small amounts of gum projecting into the lumen from the walls. In some cases the gum has taken the form of globules and in others it has formed a thin layer covering several pits. Note that the gum is absent from or at least is not apparent in the parenchyma beneath the upper vessel. × 260.

gum (figs. 2, 3, 5, and 6). The most probable explanation of the origin of this gum is that it is formed in the adjacent parenchyma cells and is forced through the pits into the ducts. There is sometimes evidence of a slight change in the affected vessel wall or in the region of the middle lamellae, but this is so restricted that it hardly seems probable that it serves as a source of much if any of the gum. The walls are never completely disintegrated and gum pockets are never formed as happens in many diseased plants. It seems more probable that sugars, starches, or other carbohydrates in solution in the cell sap in the adjacent parenchyma cells are the source of the gum.

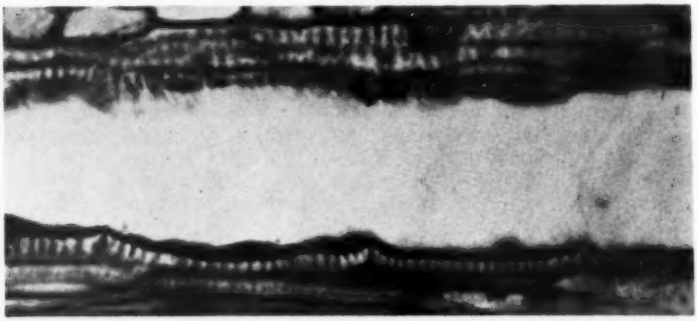

FIGURE 10.—Another vessel, from the same slide as the vessels in figure 9, showing a long sheet of gum along the wall. The gum in the early stage is semifluid so that in most cases it spreads out in a thin layer along the wall. × 300.

WATER CONTENT OF DISEASED PLANTS

The comparative amount of water in diseased and healthy alfalfa plants was determined in an experiment set up on August 20, 1931. From a field of Chilean alfalfa in its fourth year, diseased and healthy plants having about half of their blossoms open were selected. The plants all came from the same place in the field, and therefore should have been comparable in every way except for the presence of the disease in some of them. Five plants in a fairly late stage of the disease and five healthy plants were dug, wrapped well in newspaper to prevent the loss of water, and brought to the laboratory. After the adhering soil had been removed from the roots with a brush and cloth without wetting, composite samples were made of the healthy tops, diseased tops, healthy roots, and diseased roots. The roots and tops were cut into small pieces, placed in tared weighing bottles, weighed, dried to constant weight at 100° C., and reweighed. The field had been irrigated 2 days before the plants were dug, and the soil was still quite moist. A second experiment, conducted on August 25, 1931, was an exact duplicate of the first except that the tops had been cut in the meantime and the stems were only 3 to 4 inches tall and consequently were young and succulent. The soil was damp, as the field had been irrigated the preceding day. Only the new stems and leaves and about a foot of the taproots were used. This was true for both experiments.

On September 1, 1931, six diseased plants and six healthy plants from the same field were treated in the same manner as in the two preceding experiments except that the plants were held with their taproots in water for 24 hours before being prepared for the dry-weight determinations. The tops were turgid at all times. In this experiment the yellow crown tissue was placed with the roots, and all green tissue was included with the tops. The affected plants were in various stages of disease, but in all cases the disease had advanced sufficiently to cause more or less dwarfing of the tops. The healthy tops were about 1 foot to 15 inches tall. This experiment was duplicated on September 10 and again on September 16, 1931. Of course, the stage of top growth was not the same in any of these tests. On September 1 the tops were perhaps not more than half grown, while

on September 16 they were in what is usually classed as the bud stage, although there was an occasional partly open blossom.

The percentage of water in the roots and tops in these experiments is given in table 1. These data show that on each date the water content of the healthy tops was slightly higher than that of the diseased tops, while the reverse was true for the roots. The greatest difference between the moisture content of the diseased and healthy tops was on August 25, when the top growth was young and succulent. The greatest difference between the water content of the diseased and healthy roots was in the August collections. No explanation for this can be offered, unless it is the fact that the roots of the September samples stood in water before they were prepared for drying. The difference between the average percentage of water in the diseased and healthy tops and roots for all experiments is quite small and probably is not significant. Nevertheless, the tops of the healthy plants were considerably larger than those of the diseased, and it may be that the larger tops withdrew slightly more water from the roots during the period which of necessity elapsed between the removal of the plants from the soil or water and the determination of the wet weight. Perhaps the slightly lower water content of the diseased tops was due to a partial water deficit. That such a deficit does exist at times is suggested by the fact that diseased plants often wilt even when growing in wet soil.

TABLE 1.—*Average percentage of water in healthy and dwarfed alfalfa tops and roots on different dates in 1931*

Date	Average percentage of water in—			
	Tops		Roots	
	Healthy	Diseased	Healthy	Diseased
Aug. 21	70.1	68.5	69.5	74.4
Aug. 25	82.5	77.2	66.2	70.9
Sept. 1	82.2	79.2	70.5	70.7
Sept. 10	82.7	80.6	68.8	69.3
Sept. 16	77.8	75.2	67.4	70.1
Average	80.0	77.3	68.7	70.5

TRANSPIRATION

A study was also made of the rate at which dwarfed and healthy plants transpire. Twelve 4-year-old Chilean alfalfa plants, six dwarfed and six healthy, which had received the same treatment throughout, were used for each experiment. The plants were collected at about the same time in the morning (8 a. m.), wrapped well in newspaper, and taken to the laboratory, where the roots were placed at once in water. As much of the taproot as could be pulled conveniently, usually about 8 to 12 inches, was left on each plant. The length of the tops varied in the different experiments. The healthy tops ranged in height from 15 to 16, 14 to 18, and 26 to 31 inches in the experiments conducted on September 1, 10, and 16, 1931, respectively. The tops were of different ages and different stages of maturity, since they were all cut on August 24. The healthy

plants had reached the bud stage by September 16, when the last experiment was conducted. As soon as possible after the plants were brought to the laboratory the taproots and crowns were washed free of adhering soil, and after the lower end of the taproots had been cut off, taproots and crowns were placed at once in 100-cc graduated cylinders filled with tap water. Sufficient water was added to or withdrawn from the graduates to bring the water level to the 100-cc line. The graduates were placed on a table in two rows, so arranged that the tops of the plants did not touch and no two diseased or healthy plants were adjacent. In this way it was hoped to have the diseased and the healthy plants subjected to the same average conditions. The room temperature ranged from about 22° to 30° C. during the experiment, and there was some air circulation from open windows, but no wind was blowing directly upon the plants. The space between the roots and the mouth of the graduate was left open, since preliminary trials showed that not enough water evaporated from the surface in 7 hours to vitiate the results.

After the graduates had been filled as described, a measured amount of water was added each hour to bring the level back to the 100-cc mark. In this manner the total amount of water transpired by each plant in 7 hours was determined. The averages of these values, each representing the average for six plants, for the three experiments are given in table 2. Considerable variation was shown in the amount of water transpired both by the different plants and from hour to hour by the same plants. As might be expected, the greatest water loss occurred during the warmest part of the day.

TABLE 2.—*Comparative rate of transpiration by dwarfed and healthy alfalfa plants*[1]

Average amount of water transpired per plant in 7 hours		Average volume of tops		Average dry weight of tops		Average amount of water transpired in 7 hours				Ratio of diseased to healthy	
						Per cubic centimeter of green top		Per gram of dry top			
Healthy	Diseased	Healthy	Diseased	Healthy	Diseased	Healthy	Diseased	Healthy	Diseased	Volume basis	Dry-weight basis
Cc	*Cc*	*Cc*	*Cc*	*G*	*G*	*Cc*	*Cc*	*Cc*	*Cc*		
67.1	28.8	85.3	68.6	10.7	7.5	0.80	0.42	6.27	3.84	0.53	0.61
58.1	23.3	74.5	50.0	8.3	5.8	.78	.47	7.00	4.02	.60	.57
66.4	26.7	99.5	53.7	15.5	8.4	.67	.50	4.28	3.18	.75	.74

[1] 6 dwarfed and 6 healthy plants were used in each of the 3 experiments.

The data presented in table 2 show that the healthy plants transpired more rapidly than the diseased plants in all experiments. The tops, however, varied considerably in size; hence, to get the comparison on a more uniform basis, the volume of tops as well as their dry weight was determined. The volume of the tops was first obtained by the water-displacement method, after which they were dried to constant weight in an oven at 100° C. When the volume of tops is used as the criterion, a comparison of the rate of transpiration in the three experiments shows that the healthy tops transpired 0.80, 0.78, and 0.67 cc, and the diseased tops 0.42, 0.47, and 0.50 cc per cubic centimeter of tops, respectively. The corresponding values, obtained

by using the grams of dry weight as a basis of comparison, are 6.27, 7.00, and 4.28 cc for the healthy plants and 3.84, 4.02, and 3.18 cc, for the diseased plants, respectively. When the ratio of diseased to healthy plants was calculated, the values in the last column were obtained. These values show a fairly close agreement between the results obtained by the two methods. In general, they indicate that the diseased plants transpired on an average only 53 to 75 percent as fast as the healthy plants, or, expressed in another way, the healthy plants on a volume basis transpired on an average, about 1.6 times as fast per unit of top tissue, as did the diseased plants.

RATE OF WATER FLOW THROUGH DISEASED ROOTS

After it had been determined that alfalfa plants affected with dwarf transpire much more slowly than do healthy plants, the rate at which water could pass through diseased roots was studied. It is known that the tops of diseased plants sometimes wilt during the warm part of the day even when the soil is wet. This indicates that the tops cannot obtain water rapidly enough to offset that lost by transpiration. Likewise, it has been shown by microscopical examination that many of the vessels of affected plants are plugged with gum. That the slowing down of the rate of transpiration and the plugging of the vessels were somewhat interrelated seemed probable.

The rate at which the water can pass through 4-inch segments of the taproots of diseased and healthy plants was determined by a method similar to that described by Melhus, Muncie, and Ho (*3*). Water was pulled through segments of the roots of 4-year-old alfalfa plants under a constant suction pressure. The diseased and healthy plants were obtained from the same place in the field, were collected at the same time, and were held under comparable conditions throughout.

TABLE 3.—*Average amount of water pulled through 4-inch segments* [1] *of healthy and diseased alfalfa roots of different diameters in 10 minutes*

Diameter of root at small end	Healthy roots		Diseased roots		Diameter of root at small end	Healthy roots		Diseased roots	
	Plants	Average amount of water pulled through in 10 minutes	Plants	Average amount of water pulled through in 10 minutes		Plants	Average amount of water pulled through in 10 minutes	Plants	Average amount of water pulled through in 10 minutes
Mm	*Number*	*Cc.*	*Number*	*Cc.*	*Mm*	*Number*	*Cc.*	*Number*	*Cc.*
6	2	19.5	1	4.7	10	3	53.1	2	36.0
7	3	10.1	1	8.4	11	5	61.3	4	23.4
8	5	24.8	4	11.3	12	2	55.7	6	26.4
9	3	24.0	5	24.5	13	2	86.1	2	59.9

[1] Each segment was taken from a different plant.

The plants required for the day's testing were brought to the laboratory in the morning and kept in water until used. For the most part, a diseased and then a healthy plant was studied in order to minimize any error that might be due to a change in the plants on standing in water. The segment of root used was taken just below the crown. The diameter of the smaller end was used as a basis of

comparison, since it was assumed that the maximum amount of water that could pass through the root was limited to the amount that could enter the small end. The number of cubic centimeters of water that passed through the 25 segments of the diseased and the healthy roots in 10 minutes was determined, and the values are recorded in table 3. The data in this table show considerable variation in the amount of water that passed through roots of different diameters. Individual healthy roots of the same diameter seemed to vary considerably in this respect. Perhaps this is to be expected of roots of this age and character. Seldom do any two plants have crowns or tops of the same size, or indeed taproots of the same length. Taproots likewise vary greatly in diameter even in a distance as short as 4 inches.

This experiment, however, had only one object, namely, to compare diseased and healthy plants, and it was thought that if any appreciable difference existed it would be brought out by a study of 25 plants of each group. This proved to be the case, as table 3 shows. Although the differences were sometimes slight, as in the case of the roots 7 mm in diameter, for the most part they were rather striking. In the case of roots 9 mm in diameter, the healthy roots conducted more water than the diseased except for one diseased root, which conducted water more freely than any of the healthy roots and was responsible for the slightly higher average for the diseased roots. That the passage of water through the diseased plants should vary greatly in rate is to be expected, not only in view of the variation found in its passage through the healthy plants but also because of the difference in the stage of disease and consequently in the number of ducts plugged with gum. It was impracticable to use roots entirely comparable in every way, owing to the difficulty of obtaining them and to the impossibility of telling by macroscopic examination the exact amount of plugging in the diseased root. The average amount of water pulled through the healthy and diseased roots was 40.6 cc and 25 cc, respectively. This means that on an average water passed through the healthy root segments 1.6 times as fast as through the diseased roots. The fact that the healthy plants transpired approximately 1.6 times as fast as did the diseased plants and permitted the passage of water 1.6 times as fast as the latter suggests that there is a close correlation between these two factors. The data presented in this paper seem to indicate that the inability of the affected alfalfa roots to conduct water may have much to do with the dwarfing of the tops and the ultimate death of the plant.

HYDROGEN-ION CONCENTRATION AND TOTAL ACIDITY OF HEALTHY AND DISEASED PLANTS

It is a well-known fact that plants contain various acids which are more or less highly ionized. The formation of these acids appears to be closely associated with the vital activities of the plant. Anything that alters appreciably the normal processes of a plant may effect a corresponding change in the acidity of its sap. Hurd (*2*) found that the expressed juice of corn plants varied greatly in both hydrogen-ion concentration and titratable acidity, these being influenced largely by environmental factors which affected the vegetative vigor of the plants. A number of investigators have shown that the hydrogen-ion

concentration of the sap of plants can be changed by applying certain fertilizers to the soil in which they are growing.

Since the rate of growth and transpiration of the alfalfa plant are affected by the dwarf disease, it seemed quite possible that its acidity also would be affected. To test the validity of this assumption, the hydrogen-ion concentration and total acidity of healthy and diseased alfalfa plants were determined. Four-year-old alfalfa plants of the Chilean variety, which had been given comparable treatment throughout, were used. The diseased and healthy plants came from the same area in the field; those for each experiment were collected at the same time and were given the same treatment.

The tops of diseased plants, although of the same age, were not so far advanced as those of the healthy plants. In the first two experiments the tops of the latter had made a vigorous growth and were in the early bud stage, while those of the former were somewhat less advanced, the stage of advancement depending upon the stage of the disease. The method of determining the total acidity was largely that described by Haas (*1*). Twenty-five cubic centimeters of juice from diseased and 25 cc from healthy plants were obtained by grinding the tissue in a meat grinder and then pressing the juice out through cheesecloth. The samples of juice were kept in a refrigerator until ready for use. The pH values were first determined with the quinhydrone electrode and then N/20 NaOH (1 cc at first and 2 cc later) was added until a value above 8.3, the turning point for phenolphthalein, was reached. The pH values calculated from the successive potential differences thus obtained were plotted against the volume of NaOH required to produce them. From these curves the volume of alkali required to bring the reaction to pH 8.3 was determined and taken to represent the titratable acidity of the sample.

The results thus obtained are presented in table 4. It is evident from the values shown that in the first two experiments the pH, as well as the titratable acidity, of the healthy tops checked closely. This was also true for the diseased tops and roots. The chief disagreement was in the titratable acidity of the healthy roots in the second experiment. Since the second experiment was conducted on the day following the first, the plants were approximately the same age. The last experiment, however, was performed 12 days after the second, and the tops, which had been cut on October 4, were quite young. These younger tops, in the case of both the healthy and the diseased, had a higher pH value and a lower titratable acidity than the corresponding older ones used in the first two experiments. The pH values of the healthy and the diseased roots differed little from those in the other experiments. The titratable acidity of the diseased roots differed considerably, that in the third experiment requiring nearly 10 cc more alkali than did those in the two previous experiments. It is difficult to evaluate the 25.3 cc of NaOH used for the healthy roots. Since it agrees so closely with the comparable figure for the second experiment, it seems probable that the 22.8 cc in the first experiment is the variable one and that there was no appreciable change in the healthy roots because of the new growth. A comparison of the healthy and diseased tops shows that the former had slightly higher pH values than the latter. The differences are so small, however, that they probably are not significant. On the other hand, the diseased tops in each experiment had a higher titratable acidity than

the healthy tops, although that for the third experiment was identical with that for the healthy tops in the first experiment. There was almost no difference between the pH values of the healthy and diseased roots, but the healthy roots had a higher titratable acidity in the first two experiments.

TABLE 4.—*The pH and titratable acidity*[1] *of healthy and diseased alfalfa tops and roots, 1931*

Date	Tops				Roots			
	Healthy		Diseased		Healthy		Diseased	
	pH	N/20 NaOH	pH	N/20 NaOH	pH	N/20 NaOH	pH	N/20 NaOH
		Cc		*Cc*		*Cc*		*Cc*
Oct. 1	6.10	16.0	6.05	17.3	5.80	22.8	5.80	20.6
Oct. 2	6.15	16.3	6.00	17.0	6.05	26.0	5.85	24.4
Oct. 14	6.45	13.0	6.20	16.0	5.80	25.3	5.90	30.0

[1] Titratable acidity is expressed as cubic centimeters of N/20 NaOH required to bring 25 cc of juice to pH 8.3.

In general, it may be concluded that the diseased tops had a slightly higher hydrogen-ion concentration and a higher titratable acidity than did the healthy tops, that there was practically no difference in the pH values of the roots, and that the diseased roots had a lower titratable acidity than the healthy ones except in the last experiment where the reverse was true. It is also shown that the increased vegetative vigor of the tops in the third experiment resulted in decreased acidity, the greatest difference being in the healthy tops, which were growing most vigorously. Since the disease is most evident in the roots, at least in the earlier stages, and the pH value of the diseased and healthy roots is almost identical, it seems evident that the disease does not affect the pH value of the plant, the slight difference in the pH value of the tops being attributed to the difference in the stage of their development, that is, the less vigorous growth of the diseased tops is reflected in the lower pH. Whether the slight variation in the titratable acidity of the diseased and healthy roots was due to a difference in vigor or whether it signified the presence of a diseased condition is not known. Since such a great variation existed in the titratable acidity of the diseased roots between the first two experiments and the last, seemingly owing to a change in the stage of growth of the tops, it appears probable that the condition of growth also was responsible for the smaller differences between the diseased and healthy roots.

ASH CONTENT OF HEALTHY AND DISEASED PLANTS

On July 10, 1931, healthy and diseased alfalfa plants were taken from the same place in a 4-year-old stand, the tops were removed, washed in distilled water, and wiped dry. The roots were washed in tap water and distilled water. After being cleaned, the tops and roots were divided into two samples, placed in paper bags, and dried in an oven at 75° C. The dried samples were then ground fine and ashed, and the ash was calculated as percentage of dry matter. A second

set of samples was obtained on July 24, 1931, and treated in the same manner, making four samples for each date. The data obtained (table 5) show that the ash content of both the healthy tops and roots is considerably lower than that of the diseased. In fact, the healthy tops and roots had an average of only 88 and 67 percent as much ash as the diseased tops and roots, respectively.

TABLE 5.—*Ash content of healthy and diseased alfalfa tops and roots, 1931* [1]

Date of collection	Ash content [2] of—			
	Tops		Roots	
	Healthy	Diseased	Healthy	Diseased
	Percent	*Percent*	*Percent*	*Percent*
July 10	10.10	11.13	4.27	5.85
July 24	7.73	9.10	3.43	5.65

[1] The writer is indebted to Dr. A. R. C. Haas, of the California Agricultural Experiment Station for these determinations.

[2] Expressed as percentage of dry matter.

STARCHES AND SUGARS

The differences in the rate of transpiration and in ash between diseased and healthy plants show that there is an upset in the normal physiology of the alfalfa plants affected with dwarf. Further evidence of this is supplied by a study of the carbohydrate relations of the diseased plants. Although an analytical study was not made, a few observations, especially on the starch content of the affected roots, show clearly that a marked change in the carbohydrate content of the roots takes place as the disease progresses. Many diseased and healthy roots have been sectioned, stained with iodine, and examined. These studies show that in the early stages of the disease the affected roots are gorged with starch just as are the healthy roots. As the disease progresses the starch becomes less abundant, and by the time the disease is well advanced there is little or no starch left. The only observation made on the sugars of the diseased root is that some reducing sugar is present in roots from which the starch has entirely disappeared.

SUMMARY

Alfalfa roots affected with dwarf have more or less yellow color in the wood, owing largely to the presence of gum in the vessels. In the late stages of the disease there also is present a yellow stain, which diffuses into the surrounding tissues. The gum, which is similar in character to wound gum, is limited almost entirely to the vessels in the outer xylem, the other tissues being largely unaffected. In the early stages of the disease the gum is limited to a few vessels in one or more bundles in the upper part of the taproot or crown, but before the plant is killed the whole root system is involved. Gum is found in the green stems, if at all, only in the basal 1 or 2 inches. Some of the gum evidently is forced from adjacent cells into the ducts, where it appears in the form of globules or thin sheets along the inner surface of the

walls. Eventually many of the ducts become completely filled with gum and are rendered functionless.

Many bacterialike bodies are present in some of the vessels. Microchemical tests indicate that they are chemically very similar to the gum in which they are embedded and unlike bacteria in several respects.

The water content of the tops of healthy plants was slightly higher than that of diseased tops; the reverse was true of the roots. Whether this difference is sufficiently great to be significant is questionable.

Healthy alfalfa plants transpired about 1.6 times as fast as did diseased plants.

Water could be pulled through segments of healthy roots about 1.6 times as fast as through diseased roots of approximately equal diameter, indicating a close correlation between transpiration and the rate at which water could pass through the root segments.

The diseased tops had a slightly higher hydrogen-ion concentration and a higher titratable acidity than did the healthy tops, there was no difference in the pH value of the roots, and the diseased roots had a lower titratable acidity than the healthy roots except in one experiment. It seems probable that the differences in acidity between the healthy and diseased plants were due to a difference in growth.

Roots and tops affected with dwarf had a higher ash content than did healthy plants grown under similar conditions.

Qualitative tests showed that the starch in diseased roots gradually diminishes and that it disappears altogether just before the plant dies.

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INHERITANCE OF RESISTANCE TO PYTHIUM ROOT ROT IN SORGHUM[1]

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INTRODUCTION

A destructive root rot disease that attacks milo, a grain sorghum (*Sorghum vulgare* Pers.), was observed in 1926 on the Garden City Substation of the Kansas Agricultural Experiment Station at Garden City, Kans.[2] It was determined about 5 years later that the disease is caused by a pathogenic, soil-borne organism.[3] Recently this organism was identified as *Pythium arrhenomanes* Drechs.[4] The roots of diseased plants are decayed and discolored, and these symptoms usually spread to the crown and stalk. In the field the affected plants generally die and dry up before heads can be formed, and in the greenhouse they usually are killed before they are 8 or 10 inches tall (fig. 1, *A*).

Milo cannot be produced on badly infested soil. Since 1927 this so-called milo disease has been observed on milo in Texas, New Mexico, Oklahoma, and California, as well as in Kansas at localities other than those mentioned herein. Once the disease becomes established, it often spreads rapidly throughout a field. It usually occurs where milo is grown continuously or in alternate years.

The pythium root rot of sorghums has been found only on milo and darso and on hybrids involving one or both of these types. Selections of resistant strains of milo and its hybrids are showing marked resistance in the field and greenhouse.[5] [6] The distinct host reactions shown in field and greenhouse tests for resistance to pythium root rot appeared to justify studies of the inheritance of resistance to the disease, the results of which are reported here. The data presented include two independent sets of greenhouse experiments begun concurrently at Manhattan, Kans., and at Arlington Experiment Farm, Arlington, Va. (near Washington, D. C.), together with supplementary results obtained in the field at Garden City, Kans.

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[1] Received for publication Dec. 5, 1936; issued August 1937. A joint contribution from the Departments of Botany and Agronomy, Kansas Agricultural Experiment Station, and the Division of Cereal Crops and Diseases, Bureau of Plant Industry, U. S. Department of Agriculture. Contribution no. 359 from the Department of Botany and no. 261 from the Department of Agronomy, Kansas Agricultural Experiment Station. The results of the studies in Kansas were submitted by the senior author as a thesis in partial fulfillment of the requirements for the degree of Master of Science at the Kansas State College of Agriculture and Applied Science in 1935.

[2] WAGNER, F. A. REACTION OF SORGHUMS TO THE ROOT, CROWN, AND SHOOT ROT OF MILO. Jour. Amer. Soc. Agron. 28: 643-654, illus. 1936.

[3] ELLIOTT, C., WAGNER, F. A., and MELCHERS, L. E. ROOT, CROWN, AND SHOOT ROT OF MILO. (Phytopath. note) Phytopathology 22: 265-267, illus. 1932.

[4] ELLIOTT, C., MELCHERS, L. E., LEFEBVRE, C. L., and WAGNER, F. A. PYTHIUM ROOT ROT OF MILO. (Abstract) Phytopathology 26: 92. 1936.

[5] ELLIOTT, C., WAGNER, F. A., and MELCHERS, L. E. See footnote 3.

[6] WAGNER, F. A. See footnote 2.

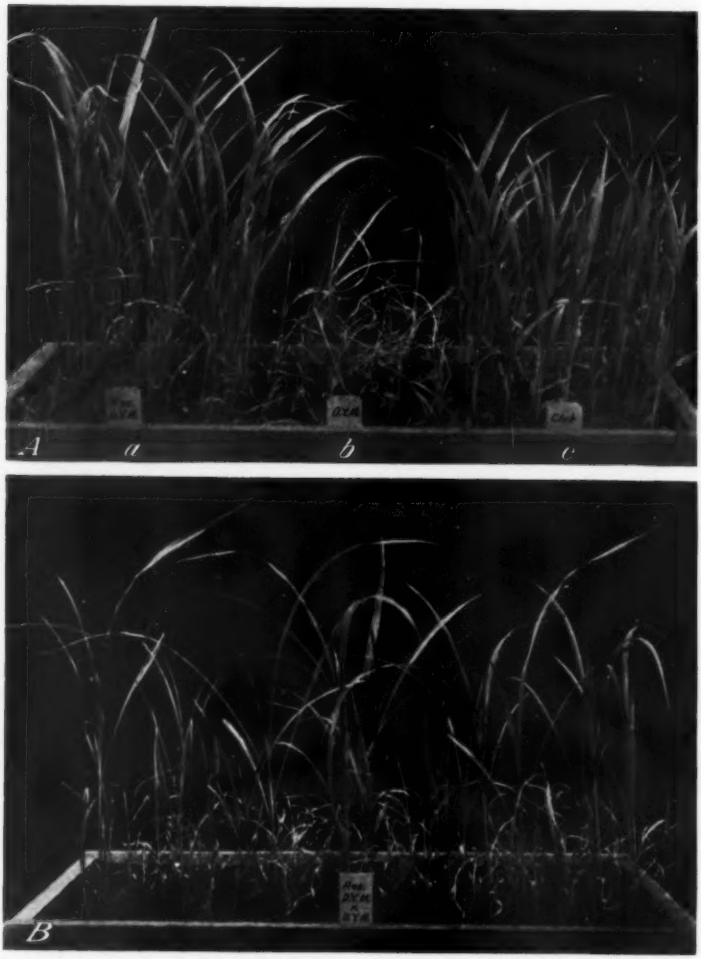


FIGURE 1.—Reaction to the milo disease of sorghum grown in infested soil in the greenhouse at Manhattan, Kans. *A*, Three parent varieties: *a*, Resistant Dwarf Yellow milo; *b*, Dwarf Yellow milo (susceptible); *c*, Club kafir (resistant). *B*, F_2 population of the cross Resistant Dwarf Yellow milo × Dwarf Yellow milo (susceptible). The tall, healthy plants are resistant segregates; the short, dead plants are susceptible to *Pythium*.

MATERIAL AND METHODS

Sorghum varieties were chosen for crossing on the basis of their reaction in nursery tests at Garden City, Kans., on infested soil. A comparison of resistant and susceptible varieties is shown in figure 1, *A*.

The susceptible varieties involved in the 12 crosses studied were Dwarf Yellow milo (C. I.[7] 332), Day milo (C. I. 959) (dwarf early

[7] C. I. refers to accession number of the Division of Cereal Crops and Diseases, formerly Office of Cereal Investigations.

type), Beaver (C. I. 871), Custer (C. I. 919), kafir × milo (No. 27–317, C. I. 963), and Early kalo (C. I. 1009). The last four were all derived from kafir × milo hybrids. The resistant varieties included Club kafir (C. I. 901), Western Blackhull kafir (C. I. 906), White kaoliang (C. I. 792), Black Amber sorgo (F. C.[8] 7038), and Resistant Dwarf Yellow[9] milo. The last-named variety was developed by F. A. Wagner from a single Dwarf Yellow milo plant that survived the disease in a severely infested plot at Garden City.

The crosses tested at Manhattan were made in the greenhouse by C. A. Wismer in the winter of 1932–33 and by D. H. Bowman and A. E. Lowe in the winter of 1933–34. The F_1 plants were grown on noninfested soil in the nursery at Manhattan in 1933 or 1934. The five crosses tested at Arlington Farm were made by John H. Martin. The F_1 plants were grown in noninfested soil in the greenhouse.

The infested soil for greenhouse experiments, which was supplied by F. A. Wagner, superintendent of the Garden City Substation, was taken from portions of the field in which the milo disease experiments had been conducted for several years, and was known to be infested. Flats were filled with the soil to a depth of about 2½ inches. Seed of the sorghum hybrids and the parental varieties was planted in rows across the flats, 8 rows to the flat at Manhattan, Kans., and 10 rows at Arlington Farm. In most of the experiments at Manhattan and in all at Arlington Farm the parental varieties and the hybrids involving them were planted in the same flat. With a few exceptions the parental varieties occupied the two outside rows of each flat. At Manhattan, three plantings were made during the winter of 1933–34 of seed from F_1 plants grown in the nursery in 1933. Each flat was thus replanted twice after final notes had been taken on the susceptibility of the previous crop and the plants had been removed. Remnant seed from the greenhouse experiment of 1933–34 was planted by F. A. Wagner on diseased soil at Garden City to supply additional data, under field conditions, on the inheritance of resistance in the F_2 generation.

In November 1934, seed was planted in the greenhouse from the F_1 plants grown in the nursery at Manhattan that season, from the additional crosses made during the previous winter. After notes on resistance had been taken the flats were replanted twice with seed from F_2 plants of the same crosses that had been tested in the greenhouse during the winter of 1933–34. The F_2 material for this third-generation study, however, had been grown on clean land in the nursery at Manhattan in 1934. Because of drought, chinch bugs, and killing frosts before maturity, seed from both F_1 and F_2 plants grown in the nursery at Manhattan in 1934 was decidedly limited and of low viability.

Three successive plantings also were made in the greenhouse at Arlington Farm from December 1933 to April 1934. By thus replanting the flats, greenhouse space could be used very efficiently for testing sorghums for resistance to pythium root rot.

At Manhattan, the seed was treated either with copper carbonate or Semesan Jr. to control seed rots before planting in the flats. The latter dust fungicide appeared to be preferable. These seed treat-

[8] F. C. refers to accession number of the Division of Forage Crops and Diseases.

[9] Since the submittal of this manuscript for publication the name of this variety has been changed to "Finney."

ments did not seem to affect the development of pythium root rot in these tests or in previous experiments by others.[10] The seed at Arlington Farm was not treated before planting, and poor stands resulted from seed rotting and from a seedling blight caused by a

FIGURE 2.—Plants from the F_2 population of the cross Resistant Dwarf Yellow milo × Dwarf Yellow milo (susceptible), grown in infested soil in the greenhouse at Manhattan: *A*, Resistant (20 plants); *B*, intermediate (15 plants); and *C*, susceptible (60 plants).

species of *Penicillium*. Susceptibility to *Pythium* and to *Penicillium* showed no association.

Symptoms of pythium root rot appeared about a month after planting and developed rapidly, so that final notes could be taken 2 to 4 weeks later. The first indication of infection in the seedling stage was the limp, ashy-gray, or seemingly scalded condition of the leaves.

[10] MELCHERS, L. E., and PARKER, J. H. CONTROL MILO DISEASE. Cappers Farmer 45: 27. 1934.

A few days later an orange tinge could be detected around the outer margin of the leaf. This color advanced over the entire plant and gradually changed to brown as the leaves dried. The youngest leaf was the last to discolor and die. Finally, the dead plants became light brown and remained upright for some time, not falling over as do plants attacked by some of the common molds.

The greenhouse temperature in the period from 1933–34 usually varied between 75° and 85° F. at Manhattan and between 70° and 80° at Arlington Farm. Duplicate plantings of nine grain sorghum varieties at two greenhouse temperature ranges, 65° to 70° and 75° to 80°, were made at Manhattan in 1934–35. This 10-degree difference in average temperature did not affect the expression of disease reaction in the varieties. Similar conclusions have been reported by others.[11]

The methods of recording the data were somewhat different in the two sets of experiments, although at both stations final counts of resistant and susceptible plants usually were made when all plants of the susceptible parent had died. At Manhattan, the plants were divided into three classes: (1) Dead (susceptible), (2) apparently diseased but not dead (intermediate), and (3) healthy (resistant) (fig. 2). Plants in class 1 could be determined easily by observation, but surviving plants were examined for general thriftiness, dead roots, and red discoloration in the central cylinder of infected roots. Plants showing lack of thriftiness and dead or discolored roots were classed as susceptible. Questionable plants were further examined by splitting the crowns. The presence of a deep reddish brown in the crown or in the subcrown internode was considered an indication that infection had taken place, as this condition was not found in healthy plants. Diseased plants were indicated also by a yellowing of the leaves. Roots arising from a discolored portion of the crown were almost always dead or dying and discolored, but any roots arising above the discolored portion of the crown often permitted the plants to survive until all of the roots became diseased. The red discoloration first appeared in the central cylinder of the root and spread later to the entire root. The dead and intermediate plants were all regarded as susceptible and are classed together in the tables that follow. Some of the roots of resistant plants showed a pinkish color, but the rest of the plant appeared healthy.

GENETIC RESULTS

THE F_1 GENERATION

One cross, Beaver milo×Resistant Dwarf Yellow milo, was tested for resistance in the F_1 generation at Arlington Farm. Only three plants emerged from the 10 seeds planted on November 11, 1933. These plants at first showed considerable resistance to *Pythium* in comparison with the susceptible Beaver parent and were still alive on December 25, when six of the eight Beaver plants were dead. By January 22, 1934, however, when the remaining Beaver plants were dead, the hybrid plants also had died. At that time the 12 plants of the Resistant Dwarf Yellow milo were still healthy. This indicated that resistance in the F_1 generation was intermediate between the

[11] ELLIOTT, C., MELCHERS, L. E., LEFEBVRE, C. L., and WAGNER, F. A. See footnote 4

parents, with a tendency toward a dominance of susceptibility. During the period of early growth it seemed that the F_1 hybrids more nearly approached the behavior of the resistant parent, but this apparent reaction did not continue.

THE F_2 GENERATION

RESULTS AT MANHATTAN, KANS.

The segregations for disease resistance in the F_2 hybrids grown at Manhattan in the seasons 1933–34 and 1934–35 are shown in table 1. The numbers of plants listed for 1933–34 are the totals for the three plantings that year. The cross Club×Resistant Dwarf Yellow milo produced all resistant plants, as might be expected from the resistance of both parents. Eight plants in a total of 392 of the Resistant Dwarf Yellow milo were classed as susceptible, but the causes of the apparent disease attack were not determined.

TABLE 1.—*Segregation for resistance to Pythium in the F_2 generation of sorghum crosses in the greenhouse at Manhattan, Kans., and at the Arlington Experiment Farm, Arlington, Va., and in the nursery at Garden City, Kans.*

Variety or F_2 hybrid	Observed			Calculated		Deviation and probable error	Deviation / Probable error
	Total	Susceptible	Resistant	Susceptible	Resistant		
	Number	*Number*	*Number*	*Number*	*Number*	*Number*	
GREENHOUSE, MANHATTAN, KANS., 1933-34							
Dwarf Yellow milo (susceptible)	426	425	1				
Resistant Dwarf Yellow milo (resistant)	392	8	384				
Club kafir (resistant)	390	0	390				
Club kafir×Resistant Dwarf Yellow milo	1,205	0	1,205				
Club kafir×Dwarf Yellow milo	1,006	728	278	754.5	251.5	26.5±9.26	2.86
Dwarf Yellow milo×Resistant Dwarf Yellow milo and reciprocal	2,402	1,732	670	1,801.5	600.5	69.5±14.31	4.86
GREENHOUSE, MANHATTAN, KANS., 1934-35							
Beaver (susceptible)	51	51	0				
Custer (susceptible)	100	100	0				
Kafir×milo, No. 27-317 (susceptible)	92	92	0				
Dwarf Yellow milo (susceptible)	168	168	0				
Resistant Dwarf Yellow milo	564	1	563				
Resistant Dwarf Yellow milo×(kafir×milo, No. 27-317)	475	6	469				
Resistant Dwarf Yellow milo×Custer	218	139	79	163.5	54.5	24.5±4.25	5.76
Resistant Dwarf Yellow milo×Beaver	1,010	727	283	757.5	252.5	30.5±9.28	3.28
NURSERY, GARDEN CITY, KANS., 1934							
Club kafir×Dwarf Yellow milo	149	107	42	111.8	37.2	4.8±3.56	1.33
Resistant Dwarf Yellow milo×Dwarf Yellow milo	736	542	194	552	184	10.0±8.02	1.24
GREENHOUSE, ARLINGTON FARM, 1933-34							
Beaver (susceptible)	12	12	0				
Beaver×White kaoliang	221	68	153	55.2	165.8	12.8±4.34	2.94
White kaoliang (resistant)	22	0	22				
Day milo (susceptible)	18	18	0				
Western Blackhull kafir×Day milo	368	120	248	92	276	28.0±4.85	5.77
Western Blackhull kafir (resistant)	34	0	34				
Club kafir (resistant)	25	0	25				
Club kafir×Day milo	346	69	277	86.5	259.5	17.5±5.43	3.22
Day milo (susceptible)	17	16	1				
Black Amber sorgo (resistant)	88	0	88				
Day milo×Black Amber sorgo	892	138	754	223	669	85.0±8.72	9.75
Day milo (susceptible)	22	13	9				

The Club kafir × Dwarf Yellow milo cross segregated according to a monohybrid ratio within the limits of random error. The cross Dwarf Yellow milo × Resistant Dwarf Yellow milo and the reciprocal of this cross (fig. 1, *B*) deviated somewhat from the 3 : 1 ratio, owing to an excess of resistant plants. The cross and reciprocal were each grown in three plantings, and in four of the six tests the observed number of resistant plants exceeded the calculated number. Although in five of the six tests the deviation from a calculated 3 : 1 ratio lay within three times the probable error, when the data are combined, as in table 1, the deviation becomes statistically significant.

At Manhattan in 1934–35 the cross Resistant Dwarf Yellow milo × (kafir × milo, No. 27–317) produced only six susceptible plants, although the latter parental variety was fully susceptible. The most plausible explanation of this is the probable difference in disease reaction between the kafir × milo plant used as the parent in the cross and the bulk variety that was tested for disease resistance. In the crosses Resistant Dwarf Yellow milo × Custer and Resistant Dwarf Yellow milo × Beaver, the number of resistant plants exceeded the calculated number by somewhat more than three times the probable error. Additional material, supposedly of the cross Resistant Dwarf milo × Custer, proved to be almost entirely resistant. This may have been due to an error in making the hybrids or to the use of a resistant plant from the susceptible Custer variety as a parent.

Seed of the cross Resistant Dwarf Yellow milo × Early kalo (susceptible) was planted at Manhattan in 1934–35, but because of poor seed only six plants emerged, and the data are not shown.

Results at Garden City, Kans.

The data obtained from two crosses grown in the F_2 generation in the Garden City disease nursery in 1934 are shown in table 1. The reactions noted were based upon an external examination of the plants in the field on October 1, 1934. In both crosses the deviation from a 3 : 1 ratio only slightly exceeded the probable error. These results, together with those obtained from the same crosses in the greenhouse at Manhattan in 1933–34, strongly suggest a single major genetic factor difference for the inheritance of resistance to *Pythium*.

The cross Club × Resistant Dwarf Yellow milo and its reciprocal also were grown at Garden City in 1934 but produced resistant plants only. This result, substantiating the behavior of this cross at Manhattan in 1933–34, indicates that the same factor for resistance is found in both parents.

Results at the Arlington Experiment Farm

Some difficulty was encountered at Arlington Farm in classifying the plants, owing to reddish discoloration of the roots possibly from causes other than *Pythium* infection. Consequently, the plants were classified at several intervals on the basis of whether they were dead or alive. Final notes usually were taken when all the plants of the susceptible parent of any particular cross were dead. At that time hybrid plants showed all gradations of development, from fully healthy to dead and dried. In two experiments at Arlington Farm,

not all the plants of the susceptible variety were dead when the final notes were taken. The condition of the susceptible parent was regarded as the most satisfactory standard for determining complete susceptibility among the hybrids. The data from Manhattan and Arlington Farm, although recorded differently, should lead to the same genetic conclusions because the difference lies merely in grouping the intermediate (or apparently heterozygous) plants with either the fully susceptible or the resistant segregates.

The data from the four crosses tested in the F_2 generation at Arlington Farm in 1933–34 are shown in table 1. The plants classed as susceptible included only those dead at the time final notes were taken. The Beaver × White kaoliang cross segregated according to

FIGURE 3.—Segregating F_2 row of the cross Western Blackhull kafir × Day milo, grown in the same flat of *Pythium*-infested soil as the parents, shown in figure 4, at the Arlington Experiment Farm.

a simple monohybrid ratio. In the Club kafir × Day milo cross the deviation from a 3:1 segregation was slightly greater than three times the probable error. In this experiment, however, one of the 17 Day milo plants was still alive when the final notes were taken. The Western Blackhull kafir × Day milo cross showed a deviation from the calculated ratio amounting to 5.77 times the probable error. The deviation was much greater in the first planting than in the second, where the ratio was very close to 3:1 (fig. 3). Both the resistant and the susceptible parent of this cross are shown in figure 4.

The test of the Day milo × Black Amber sorgo cross was of necessity discontinued before all plants of the susceptible parent, Day milo, were dead, although all plants of that variety were badly wilted and stunted. Much less than a fourth of the hybrid plants were dead at that time and those surviving showed widely varying degrees of injury.

The data as presented in table 1 indicate a wide deviation from a simple monohybrid segregation. Only 13 of the 22 Day milo parent plants in the same soil flats were dead at the time final notes were taken. If the number of dead hybrid plants is adjusted in accordance with the proportion of dead plants of the Day milo parent, the results are in close agreement with a simple monohybrid ratio.

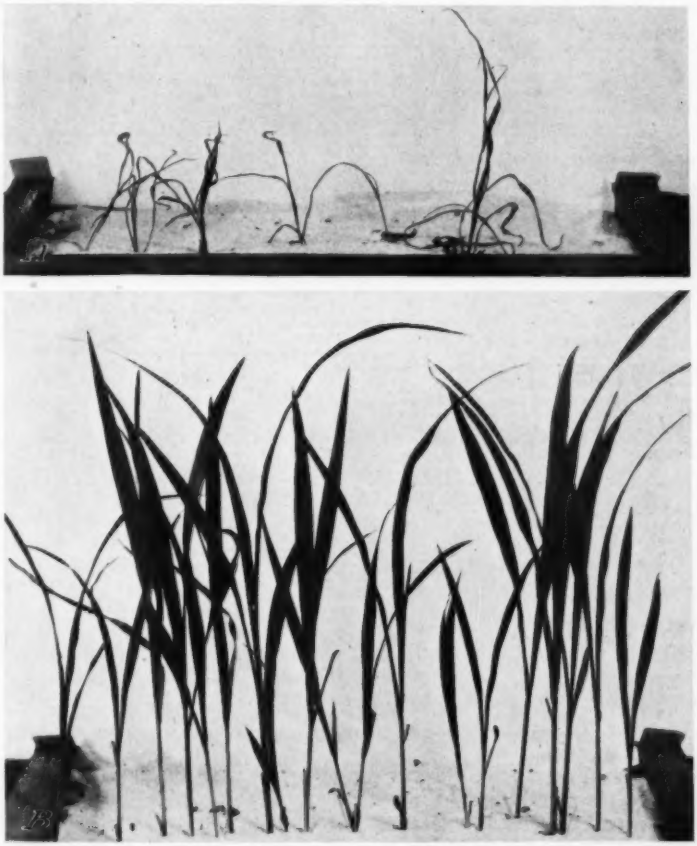


FIGURE 4.—Resistant and susceptible sorghum plants grown in the same flat of *Pythium*-infested soil at the Arlington Experiment Farm. *A*, Day milo (susceptible); *B*, Western Blackhull kafir (resistant).

THE F_2 GENERATION

The behavior of 19 F_3 lines of two crosses in the greenhouse at Manhattan, Kans., in 1934–35, is shown in table 2. Nine additional lines produced so few plants, owing to nonviable seed, that their behavior could not be determined. Considering the 19 lines of the two crosses together, 7 were classed as resistant and 12 as susceptible or segregating. The calculated numbers are 4.75 and 14.25, on the basis

of a simple monohybrid ratio, and are in rather close agreement with expected results. Each of the five segregating lines indicated a statistically significant monohybrid ratio, as did the combined results from the segregating lines of each cross. These data verified the conclusions pointing to simple inheritance that were based on data obtained in the F_2 generation of these crosses.

TABLE 2.—*Reaction to Pythium in the F_3 generation of 19 lines in two sorghum crosses grown in the greenhouse at Manhattan, Kans., 1934–35*

Cross and reaction of line	Total plants	Susceptible plants	Resistant plants	Deviation from 3:1 ratio	Deviation / Probable error
Club kafir (resistant)×Dwarf Yellow milo (susceptible):	*Number*	*Number*	*Number*		
Resistant	204	4	200		
Do	264	1	263		
Do	192	5	187		
Susceptible	41	41	0		
Do	192	192	0		
Do	252	252	0		
Do	200	200	0		
Segregating	28	20	8	1.00±1.54	0.65
Do	89	72	17	5.25±2.75	1.91
Total segregating	117	92	25	4.25±3.16	1.35
Dwarf Yellow milo (susceptible)×Resistant Dwarf Yellow milo and reciprocal:					
Resistant	78	0	78		
Do	38	0	38		
Do	31	0	31		
Do	53	0	53		
Susceptible	36	36	0		
Do	41	41	0		
Do	32	32	0		
Segregating	28	22	6	1.00±1.54	.65
Do	49	42	7	5.25±2.04	2.57
Do	68	50	18	1.00±2.41	.41
Total segregating	145	114	31	5.25±3.52	1.49

With regard to all generations of all crosses, it appears that susceptibility is partly dominant, and the segregations support the conclusion that a single major genetic factor pair largely determines the susceptibility of sorghums to pythium root rot.

COLEOPTILE COLOR AND PYTHIUM RESISTANCE

One planting of the Beaver×White kaoliang cross at Arlington Farm was classified for seedling or coleoptile color. The red coleoptile character present in the Beaver parent has been shown by Reed [12] and others to be inherited as a simple dominant character in many sorghum crosses. Small stakes were placed beside each plant having a green coleoptile, and the coleoptile color was recorded when counts of dead plants were made. The data are shown in table 3.

In this experiment the 132 plants segregated in exactly a 3:1 ratio for resistant and susceptible plants. There was a deviation of only 2 from the calculated 3:1 ratio for plants having red or green coleoptiles. When the segregations for the two characters are combined, it is seen that the number of plants in each class is very

[12] REED, G. M. A NEW METHOD OF PRODUCTION AND DETECTING SORGHUM HYBRIDS. Jour. Heredity 21: 132–144, illus. 1930.

close to the expected 9:3:3:1 ratio on the basis of independent inheritance. It appears from these results that seedling, or coleoptile, color is inherited independently of resistance to pythium root rot.

TABLE 3.—*Segregation for coleoptile color and susceptibility to Pythium in the F_2 generation of the sorghum cross Beaver × White kaoliang, in the greenhouse at the Arlington Experiment Farm, 1933–34*

Type and condition of plants	F_2 hybrid plants					Parent plants	
	Observed	Calculated (9:3:3:1 ratio)	Deviation and probable error	Calculated (3:1 ratio)	Deviation and probable error	Beaver milo	White kaoliang
Red coleoptile:	*Number*	*Number*	*Number*	*Number*	*Number*	*Number*	*Number*
Alive	74	74.25	0.25±0.47			0	0
Dead	27	24.75	2.25± .37			4	0
Total	101			99	2±3.36	4	0
Green coleoptile:							
Alive	25	21.75	.25± .37			0	7
Dead	6	8.25	2.25± .23			0	0
Total	31			33	2±3.36	0	7
Total alive	99			99	0		
Total dead	33			33	0		
Grand total	132			132		4	7

RELATION OF HETEROSIS TO PYTHIUM RESISTANCE

All the crosses tested at Manhattan, with the exception of the Resistant Dwarf Yellow milo × Dwarf Yellow milo (susceptible), showed heterosis, or hybrid vigor, in the F_1 generation, manifested chiefly by an increase in height of from 9 to 32 inches over that of the taller parent. These resistant and susceptible strains of Dwarf Yellow milo differ in resistance to *Pythium*, but they are very similar in appearance. Past experiments with sorghum hybrids have demonstrated that increased vigor is carried into the second generation.

Segregation for pythium resistance was substantially the same in Resistant Dwarf Yellow milo × Dwarf Yellow milo (susceptible) as in the other crosses in which heterosis was apparent. It would appear, therefore, that heterosis per se did not alter the inheritance of resistance.

SUMMARY

Progenies of crosses between sorghum varieties resistant or susceptible to pythium root rot (or the "milo disease"), caused by *Pythium arrhenomanes* Drechs., were studied in infested soil in the greenhouse at Manhattan, Kans., and the Arlington Experiment Farm, Arlington, Va., and in the field at the Garden City Substation of the Kansas Agricultural Experiment Station.

The data indicate that susceptibility to the disease is partly dominant. Reaction to the disease is determined by a single major factor difference.

Susceptibility is inherited independently of coleoptile (seedling) color and of hybrid vigor of the progenies.

OBSERVATIONS OF PATHOGENIC AND ANTIGENIC EFFECTS OF BRUCELLA ABORTUS, UNITED STATES BUREAU OF ANIMAL INDUSTRY STRAIN 19 [1]

By C. M. HARING, *head of division*, and J. TRAUM, *veterinarian, Division of Veterinary Science, California Agricultural Experiment Station* [2]

INTRODUCTION

Investigators at the United States Bureau of Animal Industry Experiment Station, Bethesda, Md., have reported encouraging results from the use of subcutaneous injections of living *Brucella abortus* strains of moderate or low virulence to immunize cattle against infectious abortion (Bang's disease). The first publication regarding this work at Bethesda was made by Buck (*1*)[3] in 1930, and subsequent reports have been issued by Mohler (*11*), and by Cotton (*3*). The status of these experiments in 1933 was described by Cotton and Buck (*4*), and the technical procedures and results up to 1934 were published in detail by Cotton, Buck, and Smith (*5*, *6*, *7*, *8*).

One of the cultures reported by these investigators to yield promising results in immunizing heifers against *Brucella* infection was designated by them as strain 19. They claim that this strain is fairly constant in pathogenic power as indicated by repeated tests on guinea pigs, and that it is sufficiently low in its virulence for nonlactating and nonpregnant dairy cattle to be relatively free from the objectionable feature of becoming permanently implanted in the udder following its use as vaccine.

In 1932 transplants of strain 19 were obtained by the writers from the Chief of the Bureau of Animal Industry. The following report is a summary to May 1937 on observations of its effects on guinea pigs and cattle at the California Agricultural Experiment Station. Photostatic copies of the life history for each vaccinated and control animal subjected to test exposure may be had by official investigators on application to the writers of this article.

METHODS

The methods used at the California station have conformed in general to those described in the publications by Cotton, Buck, and Smith. In transmitting cultures of strain 19 to the writers, the Chief of the Bureau of Animal Industry described these methods in a letter dated October 10, 1932, which included the following statements:

> You are advised that potato-agar slants of *Brucella abortus* strain 19, which is of moderate virulence and has given encouraging results at Bethesda, are sent you under separate cover.
>
> The method of preparing the vaccine from this culture is as follcws: The growth from 48- to 72-hour potato-agar slants is washed off with 0.85 percent salt

[1] Received for publication Sept. 16, 1936; issued August 1937.
[2] Acknowledgment is made to Mrs. Phyllis A. Zweigart, agent, U. S. Bureau of Animal Industry, for the bacteriologic procedures in this work. That bureau has also furnished laboratory and clerical assistance. The Works Progress Administration furnished assistants to care for experimental animals.
[3] References are made by number (italic) to Literature Cited, p. 128.

solution and diluted to a density of 10 times tube 1 of the McFarland's nephelometer. This is used shortly after it is prepared, usually the same day.

The animals are usually vaccinated at least 2 months before breeding and it would perhaps be better if this time were extended until agglutination reactions induced have subsided to a considerable degree. Of course, when calves are vaccinated the period is much longer.

Except for calves, 10 or 20 cc of the vaccine have been given. Seemingly, the dose may vary within considerable limits without appreciably affecting results. If more than 10 cc of vaccine are given the amount is divided between two points. Injections are usually made in front of the shoulder.

We endeavor to expose the animals when they are from 2 to 5 months pregnant. A suspension of a virulent *Brucella abortus* culture is used in making the exposures, which are by the conjunctival method. Our experiments have indicated that this method may be made to be overwhelming, therefore, we have found it important to keep it within reasonable bounds by adjusting the dosage.

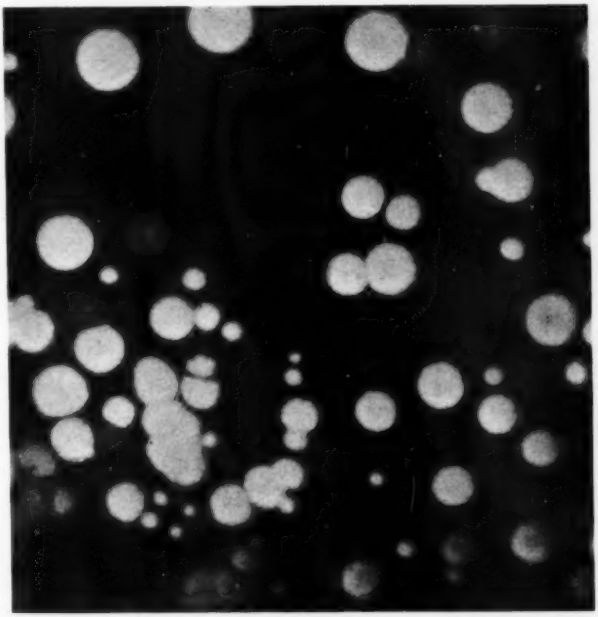
For a few months after the strain was received it was propagated on potato-agar slants, but since January 1933 Liebig's beef infusion agar, containing 2 percent of glycerin and 1 percent of glucose has been used. No change in cultural or pathogenic characteristics has been observed, and the strain has continued to conform to the descriptions published by Cotton, Buck, and Smith. When it is plated in agar, both S and R type colonies develop. In plate 1 are shown R- and S-type colonies which developed in glucose glycerin agar after being planted with a 28-day plain broth culture.

For the last 2 years strain 19 has been used at this laboratory for preparing antigens for the tube and plate methods of agglutination testing. About 80,000 tests of blood serums and whey have been made, and as yet no trouble has been experienced from self-agglutinations or other unsatisfactory effects that are supposed to be caused by the presence of R types in antigen. The precaution has been taken to occasionally renew the stock cultures from which the antigen is made by propagating from single S-type colonies.

For the isolation of *Br. abortus* from the experimental animals, the methods described by Henry, Traum, and Haring (*10*) have been followed, with the exception of the hemoculture technique which deserves special mention in view of the fact that several investigators, including the group at Bethesda, have reported difficulty in isolating *Br. abortus* from the blood stream of cattle.

Hemocultures were at first made according to the recommendations of Soule (*13*), but this proved cumbersome, laborious, and expensive, and yielded a high percentage of contaminations which rendered the method unsatisfactory. Fair success was obtained by a modification of the technique used by Shaw (*12*), which consisted in letting about 5 cc of blood run on a large cooked-blood agar slant, incubating for 6 or 7 days, stirring, and incubating again. These slants should be kept under observation for at least 2 weeks before discarding.

The best results in hemocultures were obtained by a modification of a method that has been described by Stewart, Eddie, Paxton, and Meyer (*14*). This modification consists in allowing about 5 cc of blood to run from the jugular vein into a sterile test tube in which has been placed 5 or 6 drops of a 1.6-percent solution of sodium citrate. The tube containing the citrated blood is heated in a water bath at 56° C. for 15 minutes, and 3 to 4 cc is poured over a large cooked-blood agar slant. A tube of not less than seven-eighths of an inch in diameter is used. This is incubated for 6 or 7 days in an atmosphere of 10-percent CO_2, after which the tube is tipped for a few seconds so that the blood will flow over the upper portions of the slants.



Colonies of Strain 19 on glucose-glycerin agar. This medium was seeded from a transplant in plain broth made to bring about dissociation. In this respect, Strain 19 behaves like most cultures of *Brucella abortus*. The R colonies appear white and the S colonies gray. × 15.

In this method the incubation is continued for at least 3 weeks, during which time it is examined at intervals of 3 or 4 days, and each time the tube is tipped so that the blood runs over the slant. When *Br. abortus* is present this results in an abundance of small colonies.

From a comparison of the results of blood cultured simultaneously by these different methods, it is evident that either the heating or the citrating of the blood, or both, is an important part of the technique of the latter procedure. The comparison is shown in table 1.

TABLE 1.—*Comparative results with untreated and citrated-heated blood cultures from cattle, made at various intervals after exposure to Brucella abortus*

Animal no.	Strain and method of exposure	Results from cultures of blood from jugular vein after indicated number of days following exposure [1]																							
		1		4		5		7		8		9		12		14		18		21		25		35	
		U	C	U	C	U	C	U	C	U	C	U	C	U	C	U	C	U	C	U	C	U	C	U	C
8	36 conjunctivally	−	−	−	−	..	..	co	co	..	..	..	..	−	+	..	..	..	..	−	−	..	..	−	−
9	do	−	−	−	−	..	..	−	co	..	..	..	..	−	+	..	..	..	..	−	−	..	..	−	−
10	do	−	−	−	−	..	..	−	−	..	..	..	..	−	+	..	..	..	..	−	+	..	..	−	−
1242	do	−	−	..	..	−	−	..	..	−	+	..	..	..	..	−	+	..	..	−	−	..	..	−	−
2183	do	−	−	..	..	−	−	..	..	−	+	..	..	..	..	−	−	..	..	−	−	..	..	−	−
2067	19 subcutaneously	−	−	−	−	..	..	..	..	..	..	−	−	..	..	..	..	−	+	..	..	−	−	−	−

[1] U indicates that fresh untreated blood was spread over the surface of cooked-blood agar slants; C indicates that citrated and heated blood was used in the same way. +=*Brucella* colonies observed; −=no *Brucella* colonies found; co=contaminated. Cultures were continued at fortnightly intervals for 100 days with negative results.

TESTS FOR VIRULENCE ON GUINEA PIGS

In addition to the use of direct cultures, approximately 200 guinea pigs have been inoculated with milk, uterine material, and other tissues of vaccinated cattle to test for the persistence of strain 19.

When this organism is present in the material inoculated it can usually be recovered by spleen culture on cooked-blood agar, provided the guinea pigs are killed about the sixth week after inoculation. Apparently the organisms of this strain tend to disappear from the guinea pig tissues soon after the sixth week. Macroscopic lesions in guinea pigs rarely result from the injection of milk or tissues containing only a few organisms of this strain. The agglutination titer is usually low. The absence of any apparent enlargement of the spleen or macroscopic change in the parenchyma of the spleen, liver, testes, or lymph nodes is in marked contrast to the definite lesions resulting from the inoculation of milk or tissues containing more virulent strains of *Br. abortus*.

When culture suspensions of a billion or more organisms of strain 19 are injected intra-abdominally a positive blood agglutination titer usually results and, occasionally, slight enlargement of the spleen and other slight lesions. A series of 18 guinea pigs were injected intra-abdominally with 0.2 to 0.3 cc of vaccine. Selections for autopsy from this group were made at varying intervals from 40 to 107 days. Positive cultures from the spleen were obtained in all cases up to 56 days. Two guinea pigs killed on the seventy-fourth day were negative. On the eighty-second day a third was positive and a fourth negative. Autopsies on the eighty-fourth and one hundred and

seventh day were negative. These results are in contrast to the findings in control guinea pigs inoculated with virulent strain U. C. V. 36 which yielded positive spleen cultures and definite lesions in every one of a series of 14 animals. The intervals of autopsy extended from the thirtieth to the one hundred and twelfth day.

WORK ON CATTLE WITH STRAIN 19

LONGEVITY IN BODIES OF VACCINATED CATTLE

In order to determine the longevity of strain 19 in the tissues of vaccinated cattle, and test for possible shedder conditions set up in them, a study was made of blood, subcutaneous and lymphnode tissues, uterine material, and milk. Hemocultures from the jugular veins of vaccinated calves and cows have shown that the organisms may circulate in the blood for at least 22 days following vaccination. The only instances in which the strain has been obtained from the milk or from uterine material are as follows:

Cow 2060 (table 2) discharged strain 19 in the uterine material and colostrum at the time of calving on the seventeenth day following her vaccination, but no evidence was found that she continued to shed the organisms in the milk or in the uterine material at two subsequent normal calvings.

TABLE 2.—*Results of vaccinating pregnant cows with Brucella abortus strain 19*

Group	Cow no.	Time pregnant when vaccinated	Gestation period	Description of calf	Results obtained with various materials tested for Br. abortus [1]					
					Guinea pig inoculations			Direct cultures		
					Uterine material	Colostrum	Calf tissues	Uterine material	Colostrum	Calf tissues
		Days	*Days*							
A, vaccinated while pregnant, but not otherwise exposed.	2187	28	280	Vigorous	−	−	------	------	------	------
	14	124	287	----do	−	−	------	−	−	------
	32	128	283	----do	−	−	------	−	−	------
	4	199	280	Normal (?)—found dead from exposure.	−	−	−	−	−	−
	2060	253	270	Weak	+	−	+	+	+	+[2]
B, vaccinated while pregnant and 32 days later exposed conjunctivally to strain U. C. V. 36.	2180	109	279	Vigorous	−	−	------	−	−	------
	2637	123	244	Premature; died next day.	+	−	−	+[3]	−	−
	35	128	253	Premature; lived 3 hours.	−	+	+	+	−	+[2]

[1] + = positive, − = negative.
[2] Cultures were characteristic of strain 19.
[3] Cultures were characteristic of strain 36.

Cow 35, vaccinated while pregnant 128 days, yielded strain 19 in a culture of blood from the jugular vein on the seventeenth day after vaccination, and again from the placenta and colostrum 125 days after vaccination.

Biopsy tests on a vaccinated bull have shown that the organism of strain 19 may remain alive for at least 24 days in the subcutaneous tissues and lymph nodes adjacent to the point of injection. Tests on

another bull, slaughtered on the one hundred and thirteenth day after vaccination, failed to show any living *Brucella* organisms, although direct cultures were made and several guinea pigs inoculated with a variety of tissues from various parts of the body, including the vicinity of the point of injection.

ASSOCIATION OF VACCINATED AND NONVACCINATED CATTLE

During the 3½ years that experiments with strain 19 have been in progress in Berkeley, it has been customary to keep the vaccinated cattle with approximately an equal number of nonvaccinated controls of about the same age. In addition, a few older cows have usually been in the same fields and corrals while in various stages of pregnancy. Most of the vaccinated and nonvaccinated animals have now passed through pregnancies, and inoculations of milk into guinea pigs, as well as frequent agglutination tests, have not indicated any transfer of infection from the vaccinated to the nonvaccinated cattle.

VACCINATION OF PREGNANT COWS

Cotton (*3*) and Buck (*2*) both advised against the vaccination of pregnant cows, but some veterinary practitioners venture to use culture 19 on all the animals in certain badly infected herds, regardless of the condition of pregnancy. At least one of the commercial firms manufacturing the vaccine has advocated such practice in its advertising literature.

In order to test the effects of strain 19 on pregnant cattle, a limited number of trials under the controlled conditions were made. As shown in table 2, three of the five cows vaccinated while pregnant, and not exposed, calved normally; a fourth cow gave birth to an apparently normal calf which died from exposure before being found; the fifth produced a weak calf which was killed and was the only one of the five to yield *Br. abortus*, strain 19, although at the time of each calving guinea pigs were inoculated with uterine material and colostrum.

Three other cows were vaccinated while pregnant approximately 4 months, and exposed conjunctivally with virulent *Br. abortus* U. C. V. 36 a month later. Table 2 shows that only one of these cows calved normally, with negative laboratory findings. The other two cows gave birth to premature calves which soon died, and *Br. abortus* was isolated from the placenta of each cow, the organism in one case showing characteristics of strain 19.

In certain field trials conducted by veterinary practitioners in cooperation with the writers, the vaccine has been used on cattle in various stages of pregnancy. The results so far have been variable. In one herd a rather high percentage of the cows aborted; the herd, however, was badly infected at the time of the vaccinations, and it has not been possible to determine if the abortions were due to natural infection or to injury from the vaccine.

WORK ON CATTLE WITH STRAIN U. C. V. 36

CHARACTERISTICS OF THE CULTURE USED

For conjunctival exposure trials to test the resistance of the vaccinated cattle, a strain of *Br. abortus* was selected which for 6 years has been proved repeatedly by the writers to be highly virulent for cattle. This strain, U. C. V. 36, was isolated in February 1931 in direct culture from the placenta of a cow that had aborted at a com-

mercial dairy farm. Its cultural characteristics correspond to those considered most typical of strains from bovine sources. Although transplanted at frequent intervals no tendency to dissociation has been observed in this culture. The colonies of this strain are always of the S type. It will not grow except in an atmosphere of increased carbon dioxide, and it shows the hydrogen sulphide and dye-plate characteristics of a typical bovine strain.

When 1 to 3 billion organisms of this culture are dropped upon the uninjured eye of a pregnant, susceptible cow a typical course of brucellosis follows, similar to that in a naturally infected animal in regard to blood and milk titers, abortion, and udder infection.

The subcutaneous injection of this culture into sheep during advanced pregnancy caused these animals to become shedders very soon after parturition. In some cases they continued for months to eliminate enormous numbers of organisms in their milk. In 1932 the culture was tried on three pregnant sows by intravenous inoculation of 200 million organisms and all aborted fetuses were infected with U. C. V. 36 type of organism. In three other pregnant sows, exposed orally or conjunctivally, the only evidence of infection was a rise in agglutination titer.

The culture has been used to infect 74 cattle by way of the conjunctivae, and the results are apparently satisfactory as a means of measuring the resistance of cattle to infection.

ANIMALS AND METHODS

To determine the immunity produced by vaccination, 19 vaccinated and 24 control cows have been exposed conjunctivally to virulent *Br. abortus* strain U. C. V. 36. At the time of exposure they were in various stages of pregnancy, ranging from 90 to 171 days. The results of these exposure trials are shown in table 3, which also contains a record of the reexposure, at a later pregnancy, of two of the vaccinated animals, and of two others exposed only by association with aborting cows. The animals are listed in four groups according to the respective dates of their exposure.

The suspension of strain U. C. V. 36 used for group 1 was prepared from slants made from beef-infusion agar containing 2 percent of glycerin and 1 percent of glucose. This medium was heavily seeded and incubated for 72 hours in the usual atmosphere of carbon dioxide. The surface growth was removed and diluted with physiological sodium chloride solution to a reading of 1 cm on a Gates opacimeter (McFarland tube 7). Each animal received a total of about 0.4 cc. The eyelids were then closed and gently massaged with the palm of the hand. The dose placed on the conjunctivae was estimated by plate counts to contain about 1½ billion living organisms.

The suspension of strain U. C. V. 36 used for group 2 was prepared in the same manner as that for group 1, with the exception that the medium was liver-infusion agar. The method of exposure consisted in placing 0.25 cc upon the sclera of each eyeball and a like amount under the lower lid of each eye. The lids were then closed and massaged vigorously for about 5 seconds. The total dosage given group 2 contained approximately 10 billion organisms, or about seven times the number used in exposing groups 1 and 4. Group 3 was not exposed conjunctivally, but by association with aborting animals. In exposing group 4, an effort was made to follow as closely as possible the dosage and technique of application used for group 1.

TABLE 3.—*Results of exposing vaccinated and control cattle to virulent Brucella abortus strain U. C. V. 36*

Group and how exposed to *Br. abortus*	Cow no.	Age when vaccinated	Period elapsed till exposed	Conditions at time of exposure			Blood agglutination titers[1]					Results of hemocultures on indicated number of days after exposure[2]								Parturition data				
				Total gestations completed	Age	Time pregnant	At vaccination	Highest caused	When exposed	At first positive hemoculture	Highest after exposure	1	4	7	12	21	28	35	42	Type of parturition	Gestation	Time after exposure	Cultures for *Br. abortus*	
																							Uterus	Milk
		Mos.	*Mos.*	*No.*	*Yrs.*	*Dys.*															*Dys.*	*Dys.*		
1, conjunctival exposure on Oct. 24, 1934, to 1½ billion *Br. abortus.*	1	19	24	1	3½	113	0	1,600	100	.....	400	−	−	c	−	−	−	−	−	Vigorous calf	279	166	−	−
	2	18	24	0	3½	117	0	1,600	50	.....	200	−	−	−	−	−	−	−	−	do	276	159	−	−
	3	27	24	2	4	110	0	800	−100	.....	400	−	−	−	c	−	−	−	−	do	284	174	−	−
	4	27	24	2	4	90	0	6,400	−200	.....	400	−	−	−	c	−	−	−	−	do	281	191	−	−
	5	(3)	.....	2	5	115	.....	.....	.....	.....	0	−	−	−	−	−	−	−	−	do	290	175	−	−
	6	(3)	.....	1	3	125	.....	.....	0	.....	800	−	−	−	c	−	−	−	−	Dead fetus	244	119	+	+
	7	(3)	.....	1	4	112	.....	.....	0	.....	800	−	c	c	−	−	−	c	−	do	202	90	+	+
	8	(3)	.....	1	3	92	.....	.....	0	−100	800	−	−	c	+	−	−	c	−	do	147	55	+	+
	9	(3)	.....	0	3	110	.....	.....	0	−200	1,600	−	−	c	+	−	−	−	−	do	201	91	+	+
	10	(3)	.....	0	4	114	.....	.....	50	50	3,200	−	−	−	+	+	−	−	−	do	219	105	+	+
												3	7	10	14	17	22	36	43					
2, conjunctival exposure on Oct. 29, 1935, to 10 billion *Br. abortus.*	11	4	16	0	1⅔	139	0	1,600	0	.....	3,200	−	−	−	−	c	c	−	−	Dead fetus	207	68	+	+
	12	4	12	0	1⅓	169	0	400	0	.....	−25	−	−	−	−	−	−	−	−	Vigorous calf	277	108	−	+
	13	19	36	1	4⅓	147	0	1,600	−50	−200	3,200	c	+	−	−	−	−	−	−	Dead fetus	209	62	−	+
	14	15	36	0	4¼	125	0	3,200	−100	.....	800	c	−	−	−	−	−	−	−	Weak calf	252	127	+	+
	15	15	36	1	4¼	136	0	400	0	.....	1,600	−	−	−	−	−	−	−	−	Dead fetus	222	86	+	+
	16	21	36	1	4¾	148	0	1,600	−25	.....	800	−	−	−	−	−	−	−	−	Dead calf	283	135	−	+
	17	24	12	0	3	162	0	3,200	−50	.....	1,600	−	−	−	−	−	−	−	−	Dead fetus	249	87	+	+
	18	15	36	1	4¼	.....	0	3,200	−25	.....	800	−	−	−	−	−	−	−	−	Not pregnant	.....	.....	.....	.....
	3	27	36	3	5	155	(4)	.....	50	.....	200	−	−	−	−	−	−	−	−	Vigorous calf	279	124	−	−
	4	27	36	3	5	142	(4)	.....	−200	.....	200	−	−	−	−	−	−	−	−	do	285	143	−	−
	5	(3)	.....	3	6	139	(4)	.....	0	.....	100	−	−	−	−	−	−	−	−	do	279	140	+	−
	19	(3)	.....	9	12	147	(4)	.....	−25	.....	50	−	−	−	−	−	−	−	−	do	285	138	−	−
	20	(3)	.....	1	4	157	.....	.....	0	25	12,800	−	+	+	c	−	−	−	−	Dead fetus	210	53	+	+
	21	(3)	.....	1	4	130	.....	.....	0	.....	800	−	−	−	−	−	−	−	−	Dying calf	237	107	+	+
	22	(3)	.....	0	2	164	.....	.....	0	−25	3,200	−	+	−	−	c	−	−	−	Dead fetus	209	45	+	+
	23	(3)	.....	0	2	152	.....	.....	0	.....	3,200	−	−	−	−	−	−	−	−	do	217	65	+	+
	24	(3)	.....	1	4½	169	.....	.....	200	200	6,400	−	+	+	+	−	−	−	−	do	226	57	+	+

See footnotes at end of table.

TABLE 3.—*Results of exposing vaccinated and control cattle to virulent Brucella abortus strain U. C. V. 36*—Continued

Group and how exposed to *Br. abortus*	Cow no.	Age when vaccinated	Period elapsed till exposed	Conditions at time of exposure: Total gestations completed	Conditions at time of exposure: Age	Conditions at time of exposure: Time pregnant	Blood agglutination titers: At vaccination	Blood agglutination titers: Highest caused	Blood agglutination titers: When exposed	Blood agglutination titers: At first positive hemoculture	Blood agglutination titers: Highest after exposure	Results of hemocultures on indicated number of days after exposure: 3	7	10	14	17	22	36	43	Parturition data: Type of parturition	Gestation	Time after exposure	Cultures for *Br. abortus*: Uterus	Cultures for *Br. abortus*: Milk
		Mos.	*Mos.*	*No.*	*Yrs.*	*Dys.*															*Dys.*	*Dys.*		
2. conjunctival exposure on Oct. 29, 1935, to 10 billion *Br. abortus*.—Continued	25	(2)		1	4½	171			0	−25	3,200	−	+	+	−	−	+	−	−	Dying calf	217	46	+	+
	26	(2)		1	4	166			0		800	−	−	−	−	−	−	−	−	Dead fetus	231	65	+	+
	27	(2)		4	9	165			0	25	1,600	−	−	+	−	−	−	−	−	do	223	58	+	+
	28	(2)		2	8	144			−100	−100	3,200	−	+	+	+	+	+	+	−	do	209	65	+	+
	29	(2)		1	4	152			0		1,600	−	−	−	−	−	−	−	−	Weak calf	248	96	+	+
	30	(2)		1	3½	153			0		400	−	−	−	−	−	−	−	−	do	266	113	+	−
												5	14	21	28	46	62	91						
3. in contact with aborting cows beginning Dec. 13, 1935.	31	7	17	0	2	139	0	800	0		−50	c	c	−	−	−	−	−	−	Dead fetus	186	47	−	−
	32	38	13	2	4½	131	0	1,600	−25		−100	−	−	−	−	−	−	−	−	Vigorous calf	276	145	−	−
	33	(2)		2	4½	134			0		3,200	−	−	c	−	−	−	−	−	Dead fetus	233	99	+	+
	34	(2)		0	1½				0		−100	−	−	−	−	c	−	−	−	Not pregnant				
												6	8	11	13	15	19	26	33					
4. conjunctival exposure on Nov. 6, 1936, to 1½ billion *Br. abortus*.	35	106	24	3	11	154	−100	3,200	−100		−200	−	−	−	−	−	−	−	−	Vigorous calf	286	132	−	−
	36	8	28	1	3	104	0	1,600	0		25	−	−	−	−	−	−	−	−	do	281	177	−	−
	37	39	24	2	5¼	164	−25	3,200	−200		−400	−	−	−	−	−	−	−	−	do	287	123	−	−
	38	38	24	3	5¼	117	0	3,200	−50		50	−	−	−	−	−	−	−	−	do	283	166	−	−
	39	16	28	0	3⅔	157	0	6,400	−25		6,400	−	−	−	−	−	−	−	−	Dead fetus	221	64	+	+
	40	16	28	0	3⅔	159	0	6,400	−25		3,200	−	−	−	−	−	−	−	−	do	238	79	+	+
	41	8	18	0	2⅛	115	−25	3,200	−50	−100	3,200	−	+	−	−	−	−	−	−	do	261	146	+	+
	42	(2)		0	2⅔	138			0		3,200	−	−	−	−	−	−	−	−	do	203	65	+	+
	43	(2)		0	2⅔	169			0	0	−800	+	+	+	+	−	−	−	−	do	201	32	+	+
	44	(2)		0	2½	119			0	−100	6,400	−	−	+	−	+	+	−	−	do	207	88	+	+
	45	(2)		0	2⅓	110			0	−100	6,400	−	−	+	−	+	−	−	−	do	192	82	+	+
	46	(2)		0	2	115			0	0	12,800	−	+	+	+	−	−	+	−	do	188	73	+	+
	47	(2)		0	2	167			0	0	6,400	−	+	+	−	−	−	−	−	do	220	53	+	+
	48	(2)		0	2½	163			0	−100	3,200	−	−	−	+	−	−	+	+	do	230	67	+	+

[1] Any agglutination titers shown for control animals at time of exposure were due to injections of heat-killed *Br. abortus* (bacterin) at intervals between March 1932 and June 1933.
[2] +=positive; −=negative; c=contaminated. [3] Control. [4] Second exposure. [5] Data regarding reexposed cow 19 are given on p. 125.

DISCUSSION OF TABLE 3

RESULTS WITH GROUP 1

The data for group 1 in table 3 show a marked difference in the effect of conjunctival exposure of the vaccinated cows, as compared to the controls. The only effect from exposure apparent in the vaccinated animals was a rise in the agglutination titer. It will be noted that they all gave birth to vigorous calves at full term, and the cultures of the blood, uterine material, and milk were negative. On the other hand, all but one of the controls aborted, and they also showed other effects typical of brucellosis, such as necrosis of the placenta and the presence of *Br. abortus* in the uterine material. Four controls in group 1 were proved to shed this organism in the milk and three yielded cultures of it from the circulating blood. It will be noted that control cow 5 escaped infection. During her next gestation period she was again subjected to conjunctival exposure (see group 2, table 3) and again gave birth to a full-term calf. Following her second exposure, her placenta was normal in appearance, but *Br. abortus* was isolated from it and a few weeks later her agglutination titer rose to 100. However, after a few months it subsided to negative. It is believed that she had some natural resistance to *Brucella*.

RESULTS WITH GROUP 2

There was not such a clear-cut difference between the vaccinated and the control animals in group 2, in respect to their resistance, as in group 1. This was possibly due to the fact that the cows in group 2 were exposed to about seven times the number of organisms used on those in group 1 and also to the more vigorous massaging of the eyelids in group 2. Five of the vaccinated animals developed brucellosis and the organism was isolated in each case from the placenta and milk. Of the three which failed to show evidence of the brucellosis, one was nonpregnant, one gave birth to a dead calf at full term, and only one (no. 12) produced a vigorous calf. The 11 controls (nos. 20–30), exposed for the first time, developed typical brucellosis.

In group 2 were four animals which had been previously exposed. The record of three of these (nos. 3, 4, and 5) is evident from the table, as they also appear in group 1. The fourth (no. 19) had been fed *Br. abortus* 9 years previously, when she was pregnant 114 days, which resulted in an abortion, and at that time *Br. abortus* was isolated from her placenta and milk. Thereafter her agglutination titer remained positive for 2 years, and suspicious or negative for another 2 years. The resistance shown by the four animals subjected to reexposure is so definite, in comparison with the infection of the others in this group, that one can conclude that the infection with the virulent *Br. abortus* eventually produced a much stronger resistance than did the subcutaneous vaccination with strain 19.

RESULTS OF CONTACT EXPOSURE IN GROUP 3

When the animals in this group were supposed to be 131 to 139 days pregnant, they were tested for immunity by placing them in a corral in which animals from group 2 were aborting.

One of the vaccinated cows (no. 32) gave birth to a normal, vigorous calf, and laboratory examinations for *Br. abortus* were negative.

The other (no. 31) aborted a dead fetus on the one hundred and eighty-sixth day of gestation, but, as cultures and guinea pig inoculations of uterine material, fetal tissue, and colostrum, as well as milk, were all negative for the organism, the abortion is believed to have been caused by some factor other than infection contracted through exposure to aborting cows.

One of the control animals developed an agglutination titer of —100 following contact with aborting cows, but was not found to be pregnant. The other control cow aborted a dead fetus on the two hundred and thirty-third day of gestation, and *Br. abortus* was recovered from her uterine, fetal, and udder material. The agglutination titer of this cow, which had previously been negative, rose to 1,600 at the time of abortion, in marked contrast to the low titers developed by the vaccinated cows subjected to exposure in this group.

RESULTS WITH GROUP 4

In table 3 it may be seen that group 4 consisted of seven vaccinated and an equal number of control cows. It is noteworthy that the vaccinated animals in this group that calved normally had previously borne calves, while the other three that aborted, as well as all of the controls, were primiparous.

The fact that only one of the vaccinated animals in this group yielded a positive blood culture, while six of the controls repeatedly yielded positive cultures, confirms the hemoculture results previously obtained from the animals in groups 1 and 2 and would justify the conclusion that vaccination tends to reduce the bacteraemia following exposure to *Br. abortus*.

All group 4 animals were kept continuously at pasture from the date of their weaning to the time they calved or aborted in the fall of 1936 or the early months of 1937. In this respect their treatment was similar to that given groups 1 and 2. The weather conditions during 1936, however, were more unfavorable to the nutritive qualities of the pasture vegetation than in the previous 4 years. Little rain fell between June and December 1936, and the pasture grasses (wild oats, alfilaria and bur clover) became unusually dry and bleached. Furthermore, the first fall rains were followed by such cool weather that no green forage developed until after the first of January. From November 1936 to February 1937 sparing amounts of alfalfa hay were fed two or three times a week to the animals in this group. This supplementary feeding provided a better diet than that available to the average heifer or nonlactating cow in the coast ranges of California, and at the time of exposure of group 4 the diet was considered to be adequate. It was not adequate, however, for tests by the antimony trichloride method on liver tissue from some of the fetuses showed complete lack of color reaction for vitamin A, whereas the livers of certain calves born to cows not in the experiment but kept in the vicinity and fed concentrates and all the alfalfa hay they would eat, showed a relatively high content of vitamin A.

It is probable that a combination of the effects of *Br. abortus* infection and a low intake of vitamin A, and possibly other dietary essentials, were responsible for the abortions in group 4. The results are significant as an indication of what might occur in vaccinated cattle exposed to infection under average conditions of winter range in the San Francisco Bay section.

Hart and Guilbert (*9*) have demonstrated that a lack of vitamin A in range grasses in California may cause a condition in cows resulting in the birth of dead or weak calves and retained placentas, a condition simulating infectious abortion, although the cattle may be free from brucellosis. That the pathogenic effects of *Brucella* infection may be enhanced by a lack of vitamin A in the diets of cattle is indicated by the results in group 4.

SUMMARY

Since October 1932 cultures of *Brucella abortus*, United States Bureau of Animal Industry strain 19, have been under observation. When grown on glucose-glycerin agar, the strain has apparently remained unchanged in its relatively low virulence for guinea pigs and cattle.

When grown in plain broth for about 4 weeks the strain became dissociated so that transplants to glucose agar resulted in a predominance of R-type colonies, but, when occasionally renewed by planting from S-type colonies, it remained sufficiently stable to be satisfactory for the production of antigen for the detection of agglutinins by the tube and the plate methods.

After subcutaneous injection into cattle in doses of approximately 200 billion organisms, the strain has been isolated from the circulating blood up to the twenty-second day, and from the subcutaneous tissues near the point of injection at 14 and 24 days. When injected subcutaneously into pregnant cows, it may produce typical brucellosis with transient bacteraemia, exudative and necrotic placentitis, with the discharge of large numbers of the organisms in uterine material at the time of parturition.

Cattle vaccinated with strain 19 while nonpregnant have been kept closely associated with nonvaccinated cattle for over 3 years without any evidence of the transmission of infection to the nonvaccinated animals.

The vaccination of mature nonpregnant heifers produced, for at least 2 years, a very definite resistance against the effects of exposure from introducing 1½ billion virulent *Br. abortus* into the conjunctival sacs. This resistance may be overwhelmed when larger doses are introduced into the conjunctival sacs, followed by vigorous massage of the eyelids.

Infection of adult cows with virulent strains of *Br. abortus* produced a stronger resistance to subsequent infection than was produced in younger cattle by vaccination with strain 19.

The data on results of vaccination between the ages of 4 and 16 months are too meager to permit definite interpretation, but they tend to indicate that the agglutination titer usually vanishes within a year and that only a small percentage of such animals are sufficiently protected to withstand severe conjunctival exposure when they are maintained on the low vitamin A intake available in the fall and winter months on the average California coast range.

The results from hemocultures indicate that calfhood vaccination with strain 19 tends to inhibit the bacteraemia that frequently follows conjunctival exposure of cattle with virulent *Br. abortus*.

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FURTHER STUDIES ON THE INTERRELATIONSHIP OF INSECTS AND FUNGI IN THE DETERIORATION OF FELLED NORWAY PINE LOGS[1]

By J. G. LEACH, *associate plant pathologist*, L. W. ORR, formerly *instructor in forest entomology*, and CLYDE CHRISTENSEN, *assistant forest pathologist, Minnesota Agricultural Experiment Station*[2]

INTRODUCTION

In an earlier paper[3] the authors outlined plans for a study of the interrelationships of insects and fungi in the deterioration of felled timber and described the association of two species of bark beetles with two species of blue-staining fungi. In this paper are recorded observations and experiments on several species of wood-boring beetles and their influence on the development of heart rot in felled Norway pine logs.

METHODS AND PROCEDURE

It will be necessary here only to describe briefly the general plan of the experiment. For further details the reader is referred to the earlier paper.[3] On May 18 and 19, 1931, 56 logs of Norway pine (*Pinus resinosa* Ait.), 7 to 12 inches in diameter and 40 inches in length, were cut. They were divided into 5 series of 8 logs each and a check series of 16 logs and were given the following treatments:

A. No treatment; not caged (check series).
B. Ends and limb scars disinfected with a 2-percent aqueous solution of ethyl mercury chloride, then sealed with roofing pitch and covered with burlap; not caged.
C. Same as series B, but enclosed in a cage of 18-mesh aluminum-coated screen wire.
D. No end treatment, but enclosed in a cage of 18-mesh screen wire.
E. Ends and limb scars sprayed at frequent intervals during the first two summer seasons with a 2-percent aqueous solution of ethyl mercury chloride; not caged.
F. Same as series E, but enclosed in a cage of 18-mesh screen wire.

After treatment the logs were placed in position on supports a few inches above the ground under a slanting roof of laths providing approximately 50 percent shade. The end treatments were intended to prevent fungus infection by wind-borne spores and the cages were to exclude insects and any fungi that they might introduce. The nontreated and noncaged logs of the check series were exposed to both insect attack and wind-blown fungus spores. By observing the relative amounts of decay and insect infestation in the logs of the different series it was hoped that some indication of the influence of insect infestation on the development of decay could be obtained.

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[2] The authors express their appreciation to Dr. Alexander C. Hodson for his assistance in checking the identity of the insect species mentioned in this paper and for critically reading the manuscript.

[3] LEACH, J. G., ORR, L. W., and CHRISTENSEN, C. THE INTERRELATIONSHIPS OF BARK BEETLES AND BLUE-STAINING FUNGI IN FELLED NORWAY PINE TIMBER. Jour. Agr. Research 49: 315-342, illus. 1934.

In addition to the above experiment, other logs were cut, caged, and treated as in series C and F. Into these cages varying numbers of different species of insects were introduced. In this way the development of individual species of insects and associated fungi was studied without being complicated by the presence of other insects or fungi. These served also as material for studying the various stages in the life cycle of the insects.

GENERAL OBSERVATIONS ON INSECT ATTACKS AND FUNGUS INFECTIONS

Bark beetles were the first insects to attack the experimental logs. The development of two species of these beetles and their association with certain blue-stain fungi have been described.[3] Shortly after the bark beetles had entered the logs, several species of wood-boring insects made their appearance. The most prevalent of these were the cerambycid beetles *Monochamus scutellatus* (Say), and *M. notatus* (Drury), and two buprestids, *Chalcophora virginiensis* (Drury), and *Chrysobothris dentipes* (Germar). The activities of these species and their relation to the development of wood rot in the logs are described in this paper.

In addition to these, a few other species were found in the logs in varying numbers. Since most of the latter group confined their activities to the bark and outer layers of the sapwood and made their appearance after the logs were thoroughly infested with bark beetles, no direct association with any specific fungus could be observed. A few experiments were made with some of these insects in caged and sealed logs, but no clear-cut associations with fungi were established. The most prevalent of these insects were the following:

1. *Rhagium lineatum* Oliver. This was common in most of the noncaged logs. Development was confined to the inner bark and outer sapwood. Numerous and varied species of fungi were isolated from the frass and tunnels, but no one species was consistently present, and since all the surrounding tissues had been thoroughly infested by secondary fungi, no definite associations could be detected.

2. *Hylobius congener* D. T., S., and M., a weevil, was found in several of the exposed logs. The weevils were usually near the ends of the logs and made their tunnels in the inner bark and the surface of the sapwood. Their activity was very limited and there was no consistent decay or discoloration of the sapwood associated with them. Although the frass found in the pupal chambers was frequently contaminated with numerous molds, these usually could be detected only by culturing. It is to be concluded that these insects have little or no effect on the development of fungi in the logs.

THE RELATION OF MONOCHAMUS SCUTELLATUS AND M. NOTATUS TO THE DECAY OF HEARTWOOD

Monochamus scutellatus and *M. notatus* were the most prevalent wood-boring insects found in the logs. From 2 to 15 larvae of these species were found in nearly every log that was not caged. Both species were present, *M. scutellatus* being the most abundant, but it was not always possible to distinguish the species in the larval stage. Their life histories and activities in the logs were essentially similar. For these reasons they are considered together in the following discussion. Observations and experimental data showed that the presence of these insects in the logs markedly influenced the rate of decay of the heartwood.

[3] LEACH, J. G., ORR, L. W., and CHRISTENSEN, C. THE INTERRELATIONSHIPS OF BARK BEETLES AND BLUE-STAINING FUNGI IN FELLED NORWAY PINE TIMBER. Jour. Agr. Research 49: 315-342, illus. 1934.

These species of *Monochamus* infest freshly felled pine logs or pine trees that have been killed or severely injured by some other agency. The adults are elongate, somewhat cylindrical, black or brown beetles about 1 inch in length. Individuals of each species differ considerably in size and color. The females are somewhat lighter in color than the males and are marked with irregular grayish-white spots (fig. 1, *B*).

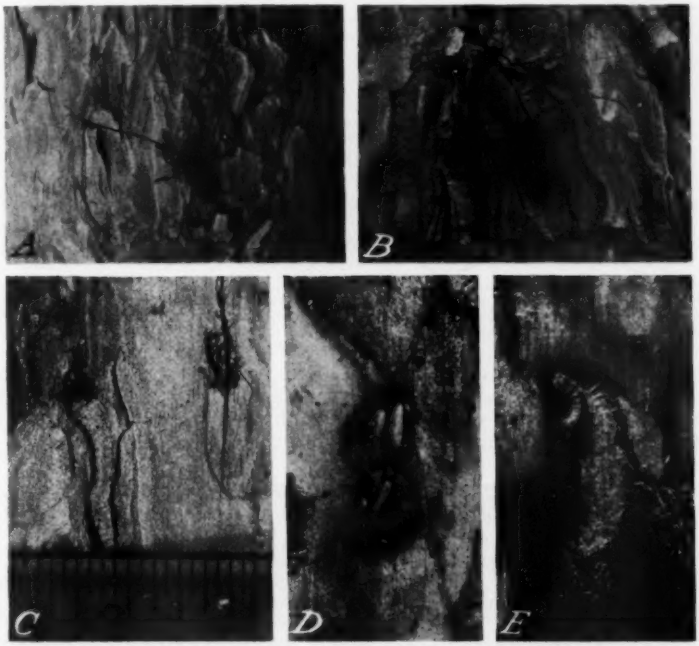

FIGURE 1.—*Monochamus scutellatus*: *A*, Male; × ½. *B*, A female in the act of chewing an egg niche in the bark of a Norway pine log; × ½. *C*, Three egg niches; × 1¼. *D*, Three *Monochamus* eggs exposed by removing the bark around a niche; × 2½. *E*, A young *Monochamus* larva feeding in the bark of a Norway pine log; × 1½.

The antennae of the males are somewhat longer than those of the females. The adults emerge from infested logs in spring and early summer. During a flight period of several weeks they feed upon the leaves or green bark of pine twigs. Oviposition reaches its peak shortly before midsummer. The eggs are deposited in slits that the females make in the bark with their mandibles (fig. 1, *C*). The slits extend into the living inner bark, and from one to three eggs are deposited in each slit (fig. 1, *D*). The larvae emerge from the eggs and tunnel in the inner bark and cambium region (fig. 1, *E*). If more than one egg hatches, usually only one larva survives, probably because of cannibalism. As the larva increases in size the excavation is enlarged and extends for a depth of a few millimeters into the sapwood (fig. 2, *A*). As the larva approaches maturity, it turns inward and bores an ovoid tunnel through the sapwood and into the heartwood for a considerable depth. Before pupation, the larva turns and bores toward the upper surface of the log. The pupal

chamber is formed about 1 inch below the surface. When the adult is formed it bores out its own emergence tunnel and emerges usually at or near the upper surface of the log through a round hole about one-quarter to three-eighths of an inch in diameter (fig. 2, *B*). The

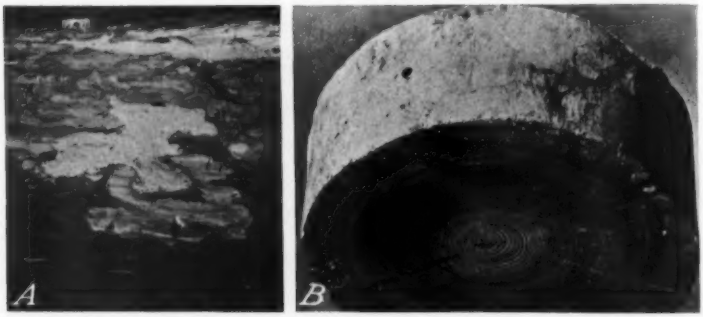

FIGURE 2.—*A*, Portion of a Norway pine log with bark removed to show feeding and penetration tunnels of two *Monochamus* larvae; *B*, section of a log (center section of log 39) showing penetration and exit tunnel of a *Monochamus* beetle. Note the decay that invaded the heartwood through the tunnel in a radial and tangential direction and spread for a considerable distance up and down the log.

time required for completion of the life cycle varies from 1 to 3 years. It is a significant characteristic of this insect that the larva tends to keep the tunnel clear of frass and chips by casting these out through holes that it makes in the bark (fig. 3, *B*).

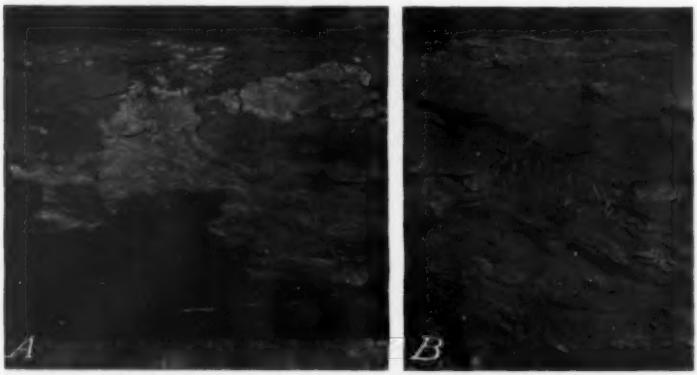

FIGURE 3.—*A*, Fruiting bodies of *Peniophora gigantea* formed on the lower side of one of the experimental logs; *B*, pile of frass pushed out of the tunnel of a *Monochamus* larva. *Monochamus* larvae keep their tunnels open by pushing all frass out through a hole made in the bark.

Graham,[4] in a study of the ecology of insects in felled logs, observed that wood rots were commonly associated with the tunnels of this insect, and suggested that the open nature of the tunnels offered ideal conditions for infection of the logs with wood rots. That there is some relation between infestations by these beetles and the decay of the heartwood is clearly demonstrated by the results of the present experi-

[4] GRAHAM, S. A. THE FELLED TREE TRUNK AS AN ECOLOGICAL UNIT. Ecology 6: 397–411, illus. 1925.

ment. In table 1 are given the summarized data on the two series of logs showing the wood-boring insects present and the corresponding amount of heartwood decay for each treatment. One of these series was examined in August 1933 and the other in September 1934, three and four summer seasons respectively after the beginning of the experiment. The insect counts were made immediately after the bark was removed from the log. The amount of heartwood decay was determined by measuring the area of decayed heartwood exposed in representative logs of each series when cut in cross or longitudinal sections, as shown in figure 4. The areas were measured with a planimeter and

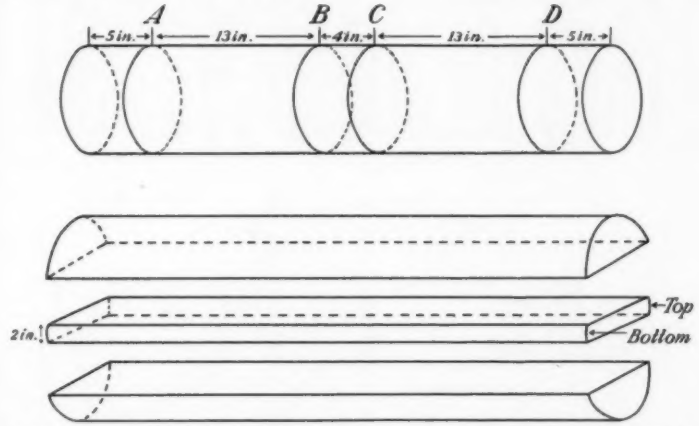

FIGURE 4.—Diagram showing how logs were cut for the measurement of the amount of heartwood decay

calculated in terms of percentage of the total area of heartwood exposed in the section. A study of the table will show that there is a fair degree of correlation between the number of wood-boring insects, especially *Monochamus* spp., and the amount of heartwood decay in the log. The correlation is shown more clearly in figures 5 and 6. It will be noted that the greatest lack of correlation is in series D and F. The logs were caged in both series. In series D the ends were given no treatment and in series F the ends were sprayed with ethyl mercury chloride at intervals during the first two summer seasons but received no treatment afterward. By the time the logs were examined the ends were badly checked, the cracks extending far into the heartwood; and it was obvious that the wood rot had spread from the sapwood deep into the heartwood through these cracks. In practically all cases in these series, as well as in series A, where the ends were exposed also, the amount of decay in the end sections was much greater than in the center sections. These differences are shown in figures 5 and 6, where the percentages of decay in the end sections are compared with those of the center sections.

The significance of the exposed ends and the deep checks that formed on the exposed ends can be more fully appreciated when they are considered in connection with the relative rate of spread of the decay in different directions in the heartwood. It was not possible to make carefully controlled measurements of the rate of spread of

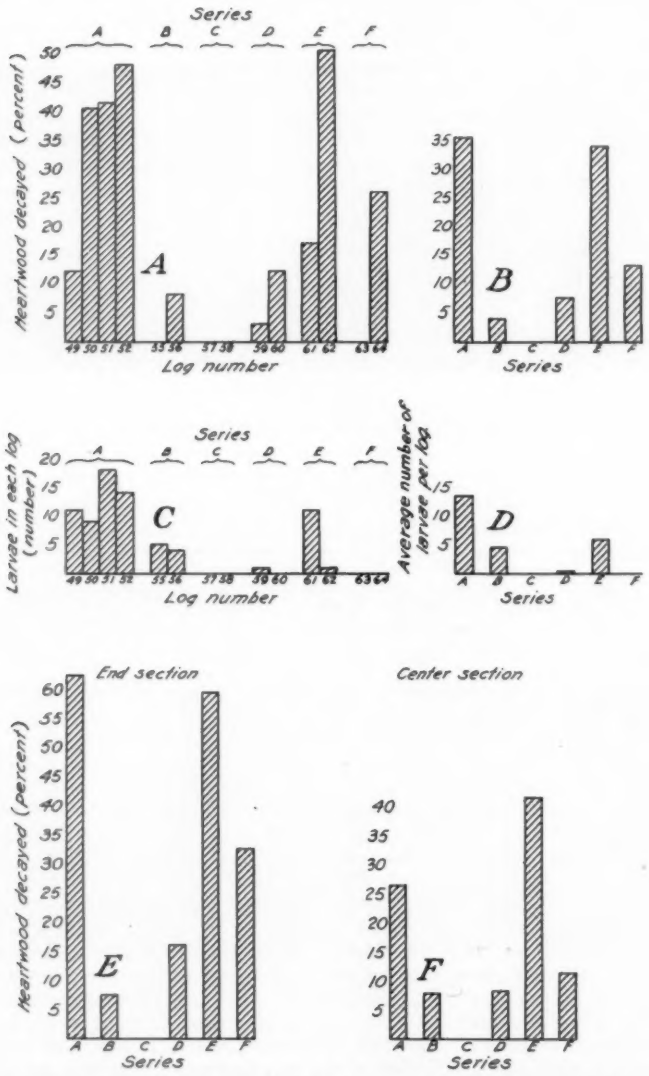

FIGURE 5.—Heartwood decay and insect infestation in logs examined after exposure for three seasons, August 1933: *A*, Total decay for each log; *B*, total decay for logs in each series; *C*, larvae of *Monochamus* spp. found in each log; *D*, larvae of *Monochamus* spp. found in logs in each series; *E*, percentage of decay in end sections of logs; *F*, percentage of decay in center sections of logs. See text for description of treatments used in each lettered series.

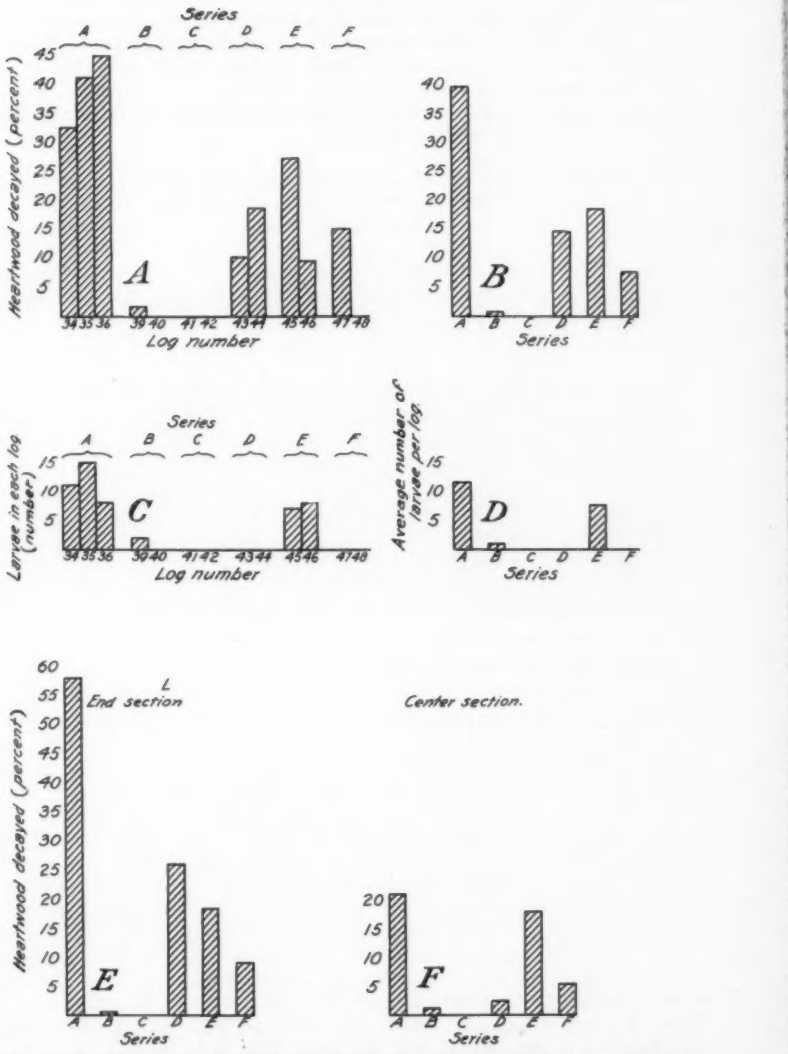

FIGURE 6.—Heartwood decay and insect infestation in logs examined after exposure for four seasons, September 1934: *A*, Total decay for each log; *B*, total decay for logs in each series; *C*, larvae of *Monochamus* spp. found in each log; *D*, larvae of *Monochamus* spp. found in logs in each series; *E*, percentage of decay in end sections of logs; *F*, percentage of decay in center sections of logs. See text for of description treatments used in each lettered series.

decay in the logs; but when the logs were cut and examined, it was perfectly obvious that the decay in the heartwood spread very much more rapidly in a longitudinal direction than either radially or tangentially. This difference was less marked in the sapwood, which was nearly always completely decayed before any appreciable decay appeared in the heartwood. Thus, in the logs with sealed ends, there was much less sapwood decay and the decay spread from the sapwood into the heartwood by radial growth very slowly except where it followed insect infestation.

TABLE 1.—*The amount of infestation by wood-boring beetles and the amount of heartwood decay in the experimental logs*

	Infestations per log																	
	Treatment A, 8 logs			Treatment B, 4 logs			Treatment C, 4 logs			Treatment D, 4 logs			Treatment E, 4 logs			Treatment F, 4 logs		
	Minimum	Maximum	Average	Minimum	Maximum	Average	Minimum	Maximum	Average	Minimum	Maximum	Average	Minimum	Maximum	Average	Minimum	Maximum	Average
	No.	*No.*	*No.*	*No.*	*No.*	*No.*	*No.*	*No.*	*No.*	*No.*	*No.*	*No.*	*No.*	*No.*	*No.*	*No.*	*No.*	*No.*
Monochamus spp.	7.0	18.0	11.6	0.0	5.0	2.7	0.0	0.0	0.0	0.0	0.0	0.0	1.0	11.0	6.7	0.0	0.0	0.0
Chrysobothris dentipes	.0	6.0	.7	.0	24.0	9.0	.0	2.0	.5	.0	1.0	.2	.0	4.0	1.0	.0	1.0	.2
Chalcaphora virginiensis	.0	.0	.0	.0	.0	.0	.0	.0	.0	.0	.0	.0	.0	1.0	.2	.0	.0	.0
Hylobius congener	.0	6.0	2.4	.0	.0	.0	.0	.0	.0	.0	13.0	6.2	.0	2.0	.5	.0	2.0	.5
Unknown cerambycid	.0	7.0	.9	.0	.0	.0	.0	.0	.0	.0	.0	.0	.0	10.0	3.2	.0	.0	.0
Unknown buprestid	.0	.0	.0	.0	.0	.0	.0	7.0	2.0	.0	.0	.0	.0	.0	.0	.0	.0	.0
Rhagium lineatum	.0	.0	.0	.0	.0	.0	.0	.0	.0	.0	.0	.0	.0	3.0	.7	.0	.0	.0
	Pct.	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*
Decay of heartwood	12.5	48.3	36.8	.0	7.9	2.4	0.0	0.0	0.0	3.0	18.5	11.0	9.2	50.5	26.0	0.0	22.2	9.3

It will be observed that the amount of heartwood decay was less, and the number of *Monochamus* beetles was smaller, in the logs with sealed ends than in those with exposed ends. Nevertheless, within the series comprising logs with sealed ends, there is a close correlation between the number of *Monochamus* beetles and the amount of heartwood decay. The greater amount of heartwood decay in the logs with exposed ends was obviously caused in part by the increased opportunity for infection through the ends. However, the moisture content of the sealed logs was much higher than that of the logs with exposed ends and probably made the conditions less favorable for both insect and fungus. The moisture content of the logs was not measured after the first year, but during the first year the average loss in weight of the logs with exposed ends was 10.69 percent, and that of the logs with sealed ends only 1.75 percent. A somewhat similar difference was probably maintained during the remainder of the period. The moisture content of the caged logs was slightly higher than that of the logs not caged, but it was not high enough to inhibit the development of decay. During the course of the experiment the progressive development of the insects and decay was studied in additional caged logs in which a known number of insects were introduced. In these caged logs the decay of the heartwood in association with *Monochamus* larvae developed equally as well as it did in the exposed logs used in the comparative test.

The principal fungus causing the decay of the heartwood was *Peniophora gigantea* (Fr.) Massee. Numerous isolations were made from decayed heartwood, including many adjacent to the tunnels of *Monochamus* larvae, in various stages of development, and this species was most frequently isolated. *Polyporus anceps* Peck, *Polyporus abietinus* (Dicks.) Fries, and *Merulius* sp. were sometimes isolated, but these were very rare. The fruiting bodies of *P. gigantea* were formed on the lower side of all logs that showed an appreciable amount of decay (fig. 3, *A*). This fungus has been recognized for a long time as the cause of sapwood decay of coniferous wood but has not been considered as seriously affecting heartwood. Although growing more slowly on heartwood than on sapwood, it is obvious from these experiments that it must also be considered as a factor in heartwood decay.

Although figures 5 and 6 show that there is a relationship between the amount of heartwood decay and infestation by the *Monochamus* beetles, the most striking proof of the association is obtained by examining the insect tunnels and the areas of heartwood decay as

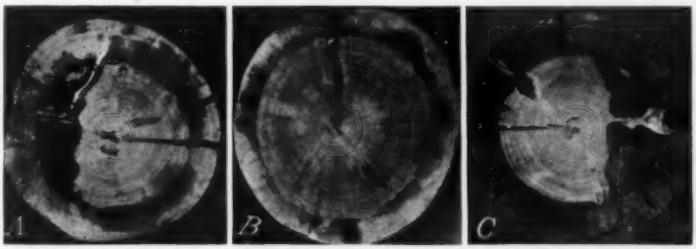

FIGURE 7.—*A*, The center section from log 45, showing two areas of decay in the heartwood that were closely associated with *Monochamus* tunnels. *B*, The center section of log 48, caged to exclude insects, showing absence of decay. In all other respects similar to log 45. *C*, The reverse side of the center section of log 45 shown in *A*.

revealed in the logs when cut in cross section. The close association of the decayed areas with the larval tunnels leaves no doubt of the fact that the decay of the heartwood is hastened by the insect infestation. This close association is well illustrated in the center sections cut from logs 39 and 45, shown in figure 2, *B*, and figure 7. The total amount of decayed heartwood resulting from each insect tunnel is not evident when seen in cross section. It is only by determining the extent of spread in a longitudinal direction that the total volume can be estimated. In log 39 (fig. 2, *B*) the decay extended more than 20 inches, and in log 45 (fig. 5) it extended the entire length of the 40-inch log.

The close association of heartwood decay with the tunnels of *Monochamus* larvae suggests the possibility that the insects may be concerned in the dissemination of *Peniophora gigantea*. If the fungus were introduced into the log by these insects it would most likely be introduced at the time of oviposition. The method of oviposition has already been described. A large number of freshly laid eggs were aseptically removed from the niches and cultured on agar. More than half of the eggs so cultured proved to be surface-contaminated with fungi. The isolations were made in 3 different years. The first year

an unidentified species of *Sporotrichum* was obtained from almost every egg. Several miscellaneous fungi were also obtained. These included *Penicillium*, *Cladosporium*, *Macrosporium*, *Fusarium*, and several kinds of yeast. In subsequent years the *Sporotrichum* was rarely found, and no other fungus was obtained with any degree of consistency. No culture resembling *Peniophora* or any other Basidiomycete was obtained.

Some of the eggs proved surface-sterile; and when the larva hatched, it also was sterile. Paraffin sections of eggs and young larvae were cut and stained, and here also no indication of internally borne fungi was found.

Cultures were made from the mouth parts and ovipositors of egg-laying females. These yielded yeasts, bacteria, and the usual assortment of molds; but no Basidiomycetes were found. Cultures made from the frass of young larvae in sound sapwood also failed to yield the wood-rotting fungus. These results led to the conclusion that these insects are of little or no importance in the dissemination of *Peniophora gigantea* or other wood-rotting fungi. *P. gigantea*, which fruits abundantly on the bark of decaying pine logs, is probably wind-disseminated, gaining access to the sapwood through holes in the bark made by insects or other means. Once under the bark, it finds the sapwood a favorable medium for growth. The sapwood is rapidly decayed, but the rate of invasion of the fungus is greatly decreased as soon as it reaches the heartwood. It can advance only very slowly in a radial or tangential direction unless aided by the larvae of *Monochamus* spp. or similar insects or by checks or cracks in the heartwood. The *Monochamus* larvae bore through the decayed sapwood and penetrate deep into the heartwood. The tunnel is a favorable place for fungus growth, and the larva, by frequent movement from one end of the tunnel to the other in pushing out its frass, is obviously a very effective aid to radial invasion by the fungus. Radial penetration having been effected, the fungus spreads rapidly through the heartwood in a longitudinal direction. It may be concluded, then, that *Monochamus scutellatus* and *M. notatus* are of little significance as agents of dissemination and inoculation of *P. gigantea*, but that in the larval stage they greatly hasten the invasion of the heartwood by the fungus.

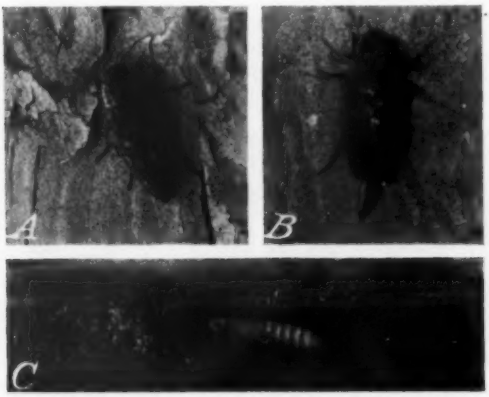

FIGURE 8.—*Chrysobothris dentipes: A*, Female on bark of a Norway pine log; × 1¾. *B*, The same in the act of ovipositing in a crevice in the bark; × 1¾. *C*, A longitudinal section through a tunnel showing pupa; × 1½. Note the frass tightly packed in the tunnel. The closed mature of the tunnel resulting from the nonremoval of frass probably accounts, in part, for the slight influence of this insect on the development of heartwood decay.

THE RELATION OF BUPRESTID BEETLES TO THE DECAY OF HEARTWOOD

It will be noted in table 1 that a considerable number of buprestid larvae were found in the logs. These consisted mainly of *Chrysobothris dentipes* (fig. 8), a smaller number of *Chalcophora virginiensis*, and several larvae of an unidentified species. There is very little correlation between the number of these beetles in a log and the amount of heartwood decayed. An examination of the tunnels made by these larvae also reveals no consistent association with the decayed heartwood.

The buprestid beetles lay their eggs in crevices under the outer bark. The eggs hatch and the young larvae bore into the inner bark and feed for most of their developmental period in the cambium region. Later they burrow into the sapwood and occasionally penetrate the heartwood but usually they do not go so deep as the *Monochamus* larvae. They develop more slowly and often do not pupate the first season; 2 or 3 years are required for completion of the life cycle.

The activity of these larvae differs from that of the *Monochamus* larvae, chiefly in the disposition of frass. This difference probably accounts, to a large extent, for the limited influence of the buprestid larvae on heartwood decay. The buprestid larvae, unlike the *Monochamus* larvae, do not remove the frass from their tunnels. The frass is packed in the tunnel behind the larvae as they advance. The tunnel is always closed and the larvae do not move to and fro in it (fig. 8, *C*). Cultures made from the frass taken from buprestid tunnels yielded a variety of yeasts and molds. No *Peniophora* or other Basidiomycete was isolated from buprestid tunnels, while *Peniophora gigantea* was abundant in the tunnels of *Monochamus* larvae.

The failure of the wood-rotting fungi to develop in the buprestid tunnels may be due to several factors. The moisture content of the frass filling the tunnels is usually very high and may be too high for good growth of the wood-rotting fungi. The wood adjacent to the tunnels also is usually rather moist. Tunnels filled with closely packed frass obviously offer less opportunity for loss of water from the adjacent wood. It is also possible that the yeasts and molds found in the frass or possibly some excretory product of the larva inhibits the development of the wood-rotting fungi.

DISCUSSION

When a Norway pine tree is felled, it immediately becomes susceptible to insect invasion. The first insects to attack the felled logs are bark beetles. As previously shown,[5] these insects introduce yeasts and certain blue-staining fungi. The life cycle of the bark beetles is short and they are found in the log only one season. Certain wood-boring beetles also attack the log soon after it is felled. The life cycle of the wood-boring beetles is longer, and these insects may be found in the logs for 2 or 3 years. During this period practically all of the sapwood and much of the heartwood of infested logs decay. No evidence has been obtained in this study to indicate that the wood-destroying fungi are introduced by the wood-boring beetles, but

[5] LEACH, J. G., ORR, L. W., and CHRISTENSEN, C. See footnote 3.

the two species of *Monochamus* do hasten the decay of the heartwood by providing a means for more rapid advance of the fungus or fungi through the wood in radial and tangential directions. The fungi, without such aid, spread very slowly in the heartwood radially and tangentially but more rapidly longitudinally. Thus the insect infestation, by hastening radial and tangential invasion, greatly increases the rate of heartwood decay. Here we have an unusual insect-plant disease relationship. The insect has no part in the usual role of dissemination or inoculation but is actively concerned with the process of invasion.

The other species of insects observed in the logs generally confined their activities to the inner bark and outer layers of the sapwood. Usually they do not appear until this region is thoroughly infested by miscellaneous fungi; they apparently have very little influence on the decay of the log.

SUMMARY

As a part of a study of the interrelation of insects and fungi in the deterioration of Norway pine logs, the changes occurring in felled logs during the second and third years after felling were studied. During this period, in fully exposed logs, the entire sapwood and a considerable portion of the heartwood were decayed. *Peniophora gigantea* was primarily responsible for the decay of both sapwood and heartwood.

No evidence was obtained to show that this fungus was dependent upon insects for dissemination or ingression. It is apparently disseminated readily by wind and may enter through cracks in the bark or through holes made by several species of insects.

The decay develops very rapidly in the sapwood but advances more slowly in the heartwood. Its spread in the heartwood is especially slow radially and tangentially but is more rapid in a longitudinal direction.

Several different species of insects were found in the logs during the period. Two species of cerambycid beetles (*Monochamus scutellatus* Say and *M. notatus* Drury) and two species of buprestids (*Chrysobothris dentipes* Germar and *Chalcophora virginiensis* Drury), were the most prevalent wood-boring beetles.

There was a fair degree of correlation between the number of *Monochamus* beetles in the logs and the amount of heartwood decayed. The larvae of these insects appear to hasten the decay by facilitating the radial and tangential invasion of the heartwood by *Peniophora gigantea*. The open larval tunnels formed by these insects are especially favorable for the spread of the decay.

The buprestid beetles have little influence on the rate of decay of the heartwood. The closed buprestid tunnels appear to be less suited for the spread of decay, and possibly this condition is largely responsible for the failure of these insects to influence the rate of decay.

Other species that inhabit the bark and outer layers of the sapwood appear to have little influence on wood decay.

STUDIES ON THE INHERITANCE AND DEVELOPMENT OF FRUIT SIZE AND SHAPE IN THE TOMATO[1]

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INTRODUCTION

The investigation described herein is concerned with the inheritance and the development of fruit size and shape in tomatoes. Locule number of the fruit, because of its apparent association with size and shape, was given special attention.

HISTORICAL REVIEW

The tomato was one of the early plants used in genetical studies after the rediscovery of Mendel's law, and shape was among the first characters mentioned. Hedrick and Booth (*4*)[3] as early as 1907 listed a shape factor. Price and Drinkard (*14*) mention round as dominant to pear shape, round-conic dominant to round-compressed, two-celled fruit dominant to many-celled, and smooth surface dominant to rough.

Various hypotheses have been advanced as to the number and relation of genes to size and shape. Frimmel (*2*) held that fruit size is complex, depending on carpel size and the degree of fasciation, with carpel size intermediate in F_1, and fasciation recessive. Lesley and Rosa (*7*) stated that oblateness is inevitable for size. Lindstrom (*8, 11*) reported genes *Pp* and *Yy* linked with a major size factor. Later (*9, 10*) he suggested as allelomorphs oval, round, and oblate linked with *Dd* and *Pp*, and reported shape and size correlated. MacArthur (*13*) gave 10 linkage groups involving 20 genes, but did not include locule number as one of these. Currence (*1*) reported significant association between fasciation and fruit size, suggesting that factors affecting fruit contour probably also affect size.

Sinnott (*15*), working with *Cucurbita*, traced the histological development of shape and size. He found shape determined in the earliest primordia of the pistillate flower, and that cell division had ceased at the time of anthesis. Houghtaling's (*5*) studies with tomato have supplied the reason for the correlations between ovary and fruit measurements reported by Hackbarth et al. (*3*) in crosses between *Lysopersicon Mill. esculentum* and *L. racemigerum*. She found that a differential rate of growth occurs at earliest primordia, ceasing in *L. esculentum* at anthesis, at which time cell division is complete.

There has been some confusion in the use of terms. The fasciation reported by Frimmel (*2*), Warren (*18*), and Houghtaling (*5*) evidently

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[2] Acknowledgment: In the pursuance of this investigation and the preparation of this manuscript, the invaluable advice and assistance of Dr. E. W. Lindstrom have been freely given and are gratefully acknowledged.

[3] Reference is made by number (italic) to Literature Cited, p. 152.

refers to an increase in the number of locules or, as Price and Drinkard (*14*) call them, cells. On the other hand, Currence (*1*) and MacArthur (*12*) refer to rough fruit without regard to locule number. Such confusion is easily accounted for by the fact that in the many-loculed fruits roughness is much more easily seen.

MATERIALS AND METHODS

The seed stocks used in this work were procured from the following sources: The department of genetics, Iowa State College; the department of horticulture, North Dakota Agricultural College; and commercial seedsmen. Fruit shape, i. e. polar and equatorial diameter ratio, was determined, unless otherwise stated, by the use of calipers. Locule number was determined by sectioning. Ovary measurements were made by the use of a low-power microscope and an eyepiece micrometer. Wherever possible, the mean of 10 fruits was used in determining fruit and ovary shape and locule number.

In this investigation the locule number will be dealt with, the suggested gene symbols *Lc* (few) and *lc* (many) locules being used, as distinct from the *Ff* (rough fruit or fasciation) genes.

For shape, *o* (oval) will be used to indicate a polar diameter 1.2 or more times the equatorial diameter, *O* (round) for a ratio of 0.95–1.2, and *O′* (oblate) for a ratio less than 0.95. *O′* is used for convenience only, inasmuch as the existence of this suggested allelomorph has not yet been proven.

Standard error rather than probable error is used. In all correlations, one asterisk (*) indicates a significant correlation and two asterisks (**) a highly significant one, as calculated from table 16 of Wallace and Snedecor (*17*).

The following known genes are involved in this study:

Dd	standard-dwarf plants.
Oo	oblate-oval fruits.
Ss	simple-compound flower cluster.
Rr	red-yellow fruit.
Yy	yellow-clear skin.
Spsp	indeterminate-determinate plant.
Cc	cut-potato leaf.
Aa	purple-green stem.
Ll	green-lutescent foliage.

EXPERIMENTAL PROCEDURE

SIZE, SHAPE, AND LOCULE NUMBER CORRELATIONS

Quantitative characters may often best be studied by determining whether they are associated with qualitative ones. It was decided to approach locule number in this way. Crosses were therefore made in which the parental stocks differed in locule number and in other characters which could be separated readily. F_2 and backcross progenies were then classified according to these, and their relation to mean locule number determined.

In table 1, under A, B, and C, such tabulated F_2 generations and backcrosses will be seen. In these it will be noted that there is a significant association between locule number and the first-chromosome-linked genes *Dd Oo Ss*, but no association with the *Rr* genes.

In B, the backcross population is divided into two classes *O* and *O'*, rather than *O* and *o*, because the plant used in test crossing was oblate, producing when crossed with *o* a round fruit, and when crossed with *O* an oblate fruit.

Populations D, E, F, and G show "dwarf" coming into the cross associated with many locules which is the reverse of the crosses in A, B, and C, but again there is no significant association with *Rr* and *Cc*. Population H involves *Yy*, and here again there is no evidence of association. This close association between genes of the first chromosome and locule number does not, however, prove the existence of any gene for locule number. Locule number might perhaps be an indirect effect of the shape gene, or of size previously reported as associated with first chromosome genes by Lindstrom (*10*).

TABLE 1.—*Association of certain tomato characteristics with locule number*

Populations [1] and parents (See p. 5)	Phenotypic segregates	Mean number of locules	Difference
A–F_2	197 *D*	3.21	0.70±0.09
(26) *D O S lc* / (3) *d o s Lc*	55 *d*	2.51	
	211 *S*	3.16	.52± .10
	41 *s*	2.64	
	134 *O*	3.33	.91± .08
	46 *o*	2.42	
B–backcross	28 *D*	4.61	.58± .11
(26) *D O R lc* / (3) *d o r Lc* × (46) *d O' r lc* / *d O' r lc*	23 *d*	4.03	
	32 *O*	4.12	.44± .06
	19 *O'*	4.56	
	18 *R*	4.36	.02± .13
	33 *r*	4.34	
C–F_2 (Bison) *O lc* / (Yellow Pear) *o Lc*	138 *O*	3.24	.68± .10
	44 *o*	2.56	
D–backcross (49) *d lc* / (9) *D Lc* × (40) *d lc* / *d lc*	45 *D*	3.90	.57± .14
	64 *d*	4.47	
E–backcross	95 *D*	4.23	.49± .19
(40) *d O' r lc* / *d O' r lc* × (49) *d O' r lc* / (9) *D o R Lc*	80 *d*	4.72	
	81 *R*	4.46	.01± .16
	94 *r*	4.47	
	96 *O*	4.26	.54± .13
	79 *O'*	4.80	
F–backcross (49) *c d lc* / (10) *C D Lc* × (49) *c d lc* / *c d lc*	32 *D*	3.48	.61± .15
	33 *d*	4.09	
	34 *C*	3.85	.08± .15
	31 *c*	3.77	
G–backcross (9) *D Lc* / (34) *d lc* × (49) *d lc* / *d lc*	59 *D*	2.45	.78± .11
	41 *d*	3.23	
H–F_2 (47) *Y Lc* / (48) *y lc*	103 *Y*	5.59	.09± .13
	32 *y*	5.68	

[1] The capital letters A–H refer to populations; the numbers in parenthesis to the strains of tomatoes used.

As a means of studying the relationship of locule number to shape and weight of fruit, correlations were calculated between these characters in several F_2 and backcross populations. These are shown in table 2. Under populations A and B the larger parental varieties have the greater number of locules. The progenies in every case exhibit highly significant correlations between size and locule number; shape $\left(\frac{\text{equatorial diameter}}{\text{polar diameter}}\right)$ and locule number; and shape and weight. This is true even under A where the large many-loculed parental variety has oval fruits. No explanation for this is offered at this

point. It is mentioned merely to emphasize the point that there is a strong tendency for many-loculed fruits to be oblate.

TABLE 2.—*Correlations*[1] *between locules, weight, and shape in various* F_2 *and backcross populations*

Population and number of plants	Pedigree	Weight and locule number	Weight and shape	Locule number and shape
A–207	(Ohio Red) large, oval *lc* / (Yellow Cherry) small, round *Lc* F_2	**0.76	**0.30	**0.42
B–76	(Oxheart) large *lc* / (Yellow Cherry) small *Lc* F_2	**.51	**.37	**.47
C–28	(9) large *o Lc* / (36) small *O lc* F_2	.32	.06	**.85
D–47	(9) large *o Lc* / (36) small *O lc* × (40) large *O lc* / large *O lc*	.17	.00	*.31
E–15	(10) large *o Lc* / (36) small *O lc* × (40) large *O lc* / large *O lc*	.15	*.58	.46

[1] *=significant correlation; **=highly significant.

Data from crosses between comparatively large tomatoes with few locules and smaller many-loculed ones would seem to afford some possible clue to the causal relationship between size, shape, and locule number. Small, many-loculed varieties not being available an attempt was made to produce them. Crosses were therefore made between Bison, a large, many-loculed oblate tomato, and Red Currant, a few-loculed, round one; and also between Bison and Yellow Cherry. These, carried through several generations, did give pure oblate strains with many locules which had smaller fruits (42 g.) than those of the largest two-loculed oval varieties (56 g.). The synthesis of such a line indicates that locule number is not a mere secondary effect of size. These synthesized lines did not, however, have smaller locules than those of the small two-loculed parent but carried a larger number of similar sized locules and the fruits were therefore not as small. Likewise, the large two-loculed variety, while very large for a two-loculed sort, did not equal the weight of the common, many-loculed, large fruits except in weight per locule.

Under C in table 2 are found the F_2 results of the cross between a small, many-loculed and a large, few-loculed tomato. In determining shape correlations the ratio of $\left(\frac{\text{equatorial diameter}}{\text{polar diameter}}\right)$ was used as a a shape index, with classes from 0.60 to 1.80 at 0.10 intervals. Under D and E are found the F_1 results of such a cross backcrossed to large, many-loculed forms. It may be noted that while correlations exist, they are small in the case of weight and shape, except under E where the population is small and the correlations are not highly significant. The correlations between locules and weight are likewise not significant under C, D, and E where the smaller parent is many-loculed. This is in contrast to A and B where the large-fruited parent is many-loculed and where such correlations are highly significant.

Partial correlations may be used in studying the relation between size, shape, and locule number. Such correlations as those presented in table 3 show that when the parental *lc* stock is the smaller-fruited the correlation between shape and locule number is practically

unchanged if weight be eliminated; that the correlation between locules and weight likewise remains about the same when shape is eliminated, but that the correlation between weight and shape becomes negative when locule effect is removed, thus indicating that weight and shape are associated because of their mutual association with locule number. These data throw much doubt on the possibility of oblate shape being the cause of large size. A possible hypothesis would be that the small locules in the oblate parent tend to remain associated with oblateness in the progenies and to cause the oblate tomatoes in the F_2 and test crosses to be smaller, while the many locules carried by the same parent tend to increase the size of tomatoes possessing this character.

Lindstrom (*8*) concluded that locule number did not appreciably influence fruit weight, with equatorial diameter held constant. Since, however, equatorial diameter itself is a measure of size and perhaps also partly the effect of locule number, the shape index is used in this study instead of equatorial diameter. While the data of table 3 show significant correlations between locule number and weight, these are not so large as those between locule number and shape. This phenotypic association of locule number and shape has led to the work on the inheritance of locule number set forth later in this paper.

TABLE 3.—*Simple and partial correlations between* [1] *number of locules, weight and shape in F_2 populations and backcrosses involving small oblate many-loculed and large oval 2-loculed tomato varieties*

Population, number of plants	Pedigree	Locules and shape	Locules and shape (weight eliminated)	Locules and weight	Locules and weight (shape eliminated)	Weight and shape	Weight and shape (locules eliminated)
A-87	(10) large $\frac{Lc\ o}{Lc\ O}$ × (36) small $\frac{lc\ O}{lc\ O}$ F^2	**0.84	**0.82	**0.47	**0.41	**0.31	−0.16
B-44	(9) large $\frac{Lc\ o}{Lc\ O}$ × (35) small $\frac{lc\ O}{lc\ O}$ F^2	**.65	**.55	**.68	**.59	**.41	−.04
C-28	(9) large $\frac{Lc\ o}{Lc\ O}$ × (36) small $\frac{lc\ O}{lc\ O}$ F^2	**.85	**.91	.32	**.70	−.06	**−.66
D-203	(9) large $\frac{Lc\ o}{Lc\ O}$ × (36) small $\frac{lc\ O}{lc\ O}$ × (50) $\frac{\text{large } lc\ o}{\text{large } lc\ o}$	**.70	**.79	**.49	**.65	.03	**−.50
E-70	(10) large $\frac{Lc\ o}{Lc\ O}$ × (35) small $\frac{lc\ O}{lc\ O}$ × (50) $\frac{\text{large } lc\ o}{\text{large } lc\ o}$	**.88	**.92	**.62	**.69	**.36	**−.50
F-47	(9) large $\frac{Lc\ o}{Lc\ O}$ × (36) small $\frac{lc\ O}{lc\ O}$ × (40) $\frac{\text{large } lc\ O}{\text{large } lc\ O}$	*.31	*.31	.17	.18	.00	**−.60
G-58	(15) $\frac{lc\ o}{Lc\ O}$ × (15) $\frac{lc\ o}{lc\ o}$	**.79	**.79	**.69	**.69	**.38	**−.85

[1] See footnote 1, table 2.

Figure 1 depicts the distribution of F_2 and backcross populations from crosses between few-loculed and many-loculed plants. In both cases there are bimodal distributions with the low point falling at 3.5 locules. This, together with the fact that in crosses between few-loculed varieties and many-loculed ones the F_1 is two- to three-loculed, suggests this as a natural point of division between *lc* (many-loculed) and *Lc* (few-loculed). In the case of the F_2, approximately three-fourths (133) of the population would thus be classed as *Lc* and one-fourth (49) as *lc*, which fits a Mendelian 3:1 ratio with $P=0.74$. In the backcrosses the division of the population above and below this point is in the proportion of 1:1, namely 84:83. A single major gene for locule number is therefore proposed.

If locule number is due to a gene *lc*, the production of an *o lc* tomato should be possible. Steps were taken to synthesize such a variety and the outcome was successful. In the meantime a pure variety (no. 15) evidently having the same combination was selected from a strain from J. W. Lesley of California. This was used because it also carried the recessives *d* and *s*. This form, carrying genes for oval with many locules, had phenotypically round fruit, according to hypothesis, because the added locules offset the phenotypic effect of *o*. If this tomato were crossed with one carrying *Lc* and *O*, the F_2 should, if the hypothesis is correct and linkage not too great, give some plants which would be *o Lc*, and the fruit therefore oval. This procedure was followed and oval-fruited plants appeared as expected.

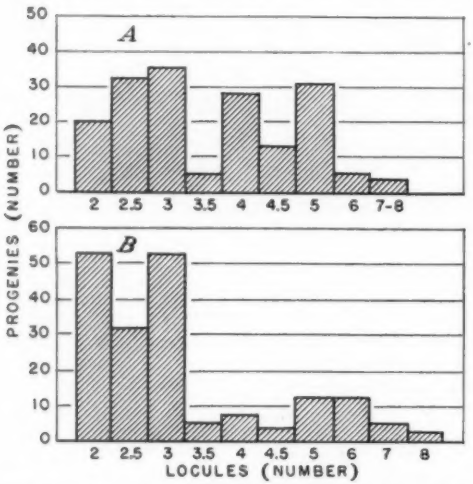

FIGURE 1.—Segregation of progenies according to locule number. Of the parental stocks used Sunrise, Yellow Pear, and Red Pear were two- to three-loculed, 15 and Bison were four- to five-loculed. Locule numbers were obtained by securing the means of 10 fruits on each plant which accounts for plants classified as having 2.5 locules per fruit, etc.

A, Combined $\frac{15}{\text{Sunrise}} \times 15$ and $\frac{15}{\text{Red Pear}} \times 15$ (backcrosses);

B, $\frac{\text{Bison}}{\text{Yellow Pear}}$ F_2.

A backcross between the F_1 and *dd, oo, ss, lclc* gave similar results. Here oval, two-loculed tomatoes appeared from parents both of which were phenotypically round.

Assuming that the round, many-loculed tomato (no. 15) did carry *oo* but, because of many locules was phenotypically round, and that many locules are recessive to few, a cross between it and an *LcLc oo* tomato should give in the F_1 not a round but an oval, few-loculed fruit. Such a cross was made and it fulfilled expectations. The F_2 and backcross populations disclosed no 2-loculed, round-fruited plants nor any many-loculed ovals, but as the number of locules increased the shape changed to oblate.

Data have already been presented in table 1 which suggest linkage between locule number and first-chromosome genes. Table 4 gives data showing the amount of this linkage with *Ss* and *Dd*. These furnish a basis for the location of *Lclc* on the chromosome map. Each population shows linkage between *Lclc* and *Ss* with a mean cross-over percentage of 20.5. Between *Lclc* and *Dd*, however, there is a mean cross-over percentage of 46.9. From these data it would seem safe to suggest that there is a gene for increased locule number (*lclc*) linked with one for compound cluster (*ss*) and that its locus is on the opposite end of the chromosome from *Dd*. With a 20.5 percent cross-over value between *Lclc* and *Ss*, as calculated here, and more than 30 units between *Dd* and *Ss* according to MacArthur, the linkage between *Dd* and *Lclc* should be very slight, as the figures in table 4 show.

TABLE 4.—*Linkage relations of lc to s and d*

Parentage [1]	Progeny				Cross-over value	X^2 2rn	P [2]
	S Lc	*S lc*	*s Lc*	*s lc*			
$\frac{(52)\ S\ Lc}{(15)\ s\ lc}$ F_2	48	3	5	9	0.142±0.026	25.7	0.01—
$\frac{(Sunrise)\ S\ Lc}{(15)\ s\ lc} \times (15) \frac{s\ lc}{s\ lc}$	42	12	14	36	.250± .034	29.3	.01—
$\frac{(Sunrise)\ S\ Lc}{(15)\ s\ lc}$ F_2	102	24	15	27	.248± .039	37.89	.01—
$\frac{(26)\ S\ lc}{(3)\ s\ lc}$ F_2	152	59	37	4	.325± .056	6.02	.02
$\frac{(Red\ Pear)\ S\ Lc}{(15)\ s\ lc} \times (15) \frac{s\ lc}{s\ lc}$	23	5	4	25	.155± .043	27.5	.01—
$\frac{(Red\ Pear)\ S\ Lc}{(15)\ s\ lc}$ F_2	104	5	10	20	.106± .028	61.47	.01—

Parentage	Progeny				Cross-over value	X^2 2rn	P
	D Lc	*D lc*	*d Lc*	*d lc*			
$\frac{(Sunrise)\ D\ Lc}{(15)\ d\ lc} \times (15) \frac{d\ lc}{d\ lc}$	30	24	26	23	0.478±0.05	0.063	0.80
$\frac{(Sunrise)\ D\ Lc}{(15)\ d\ lc}$ F_2	91	43	26	8	.561± .05	1.313	.25
$\frac{(26)\ D\ lc}{(3)\ d\ Lc}$ F_2	137	60	52	3	.240± .03	9.820	.01—
$\frac{(Red\ Pear)\ D\ Lc}{(15)\ d\ lc} \times (15) \frac{d\ lc}{d\ lc}$	14	22	14	8	.620± .06	3.566	.06
$\frac{(Red\ Pear)\ D\ Lc}{(15)\ d\ lc}$ F_2	82	16	32	9	.448± .06	.322	.60

[1] Number in parentheses refers to strain numbers.
[2] *P* value indicates the probability of differences being due to chance.

OVAL FRUITS NOT ALWAYS DUE TO THE *o* GENE

In the study of inheritance of size and shape the Ohio Red variety was used because it is large in size, nearly oval in shape, and each fruit carries several locules. One of the first crosses made with this variety was with a tomato carrying *dd rr oo LcLc*. In the F_2 from such a cross 64 plants among a total 256 were classed as oblate, and 75 as round. The appearance of oblate-fruited plants in the F_2 in crosses between these two oval varieties would indicate that the two ovals are not due to the same gene. Inasmuch as there was some possibility that the Ohio Red used in this cross was not genetically pure, another cross was made between Yellow Plum and a new stock of Ohio Red secured from the Department of Genetics of Iowa State College which had been carried through several generations and found pure. Here again 6 of the 47 plants carried decidedly oblate fruits and 5 were round.

Further evidence for the belief that the large oval differs from *o* is found in the fact that while crosses between *oo* tomatoes and *OO* two-loculed varieties produce an oblate F_1, Ohio Red in such cases gives an intermediate slightly oval $\left(\frac{L}{W}=1.1\right)$.

In F_2 populations it was found impossible to fit the progeny to a simple Mendelian hypothesis. Multiple factors are therefore

suggested. Attempts to detect linkage with the genes *Dd*, *Rr*, *Yy*, *Aa*, *Ll*, *Cc*, and *Spsp* failed, with the exception of *Dd*, and even this was inconclusive, and may have been due to the association of *Dd* with differences in locule number present in the parent stocks.

THE DEVELOPMENT OF SHAPE

In connection with the study of the inheritance of shape and size in tomatoes, measurements were made of the length and width of the ovaries at the time of anthesis, and these were compared with similar measurements of mature fruits from the same plants. The correlations between such measurements are shown in A of table 5 for an F_2 population of a cross between Yellow Pear, small-oval; and Bison, large-oblate. B gives similar figures for a group of plants of many varieties, excluding Ohio Red and Oxheart. C shows a cross involving Ohio Red. The correlation between the shape of ovary and the shape of the mature fruit in A and B is very striking, so great in fact that it seems probable that with ordinary varieties such measurements might be a better indication of the actual genetical constitution of the plant than the measurement of mature fruits, since the amount of seed and growing conditions have considerable effect upon the shape of the fruit when mature.

TABLE 5.—*Correlations between ovary measurements at anthesis and mature fruit measurements*

Population	Plants	Ovary and fruit correlations [1]		
		Length	Width	Ratio $\frac{L}{W}$
	Number			
A — $\frac{\text{(Yellow Pear) } o}{\text{(Bison) } O}$ F_2	179	**0.594	**0.953	**0.923
B — Miscellaneous varieties	78	**.619	**.639	**.903
C — $\frac{\text{(53) } O}{\text{(Ohio Red) large-oval}}$ F_2	61	.038	**.694	**.598

[1] See footnote 1, table 2.

These data are in line with those of Hackbarth et al. (*3*) who found a high degree of correlation between size of tomato ovaries and mature fruits. Houghtaling (*5*) explained this as the result of a differential growth rate which exists from earliest primordia until anthesis by which time cell division is completed. Increase in size thereafter is due to an increase in cell size. Contrasted to this is the condition in *Cucurbita* as reported by Sinnott and Kaiser (*16*), where differences in fruit shape exist at earliest primordia, and in *Capsicum* where differences appear after anthesis. A condition similar to that in *Cucurbita* was also reported in cucumbers by Hutchins (*6*) and in watermelons by Weetman (*19*).

With large-oval it will be noted (C in table 5) that length correlation is not significant. Measurements of the developing ovaries of Ohio Red revealed that at anthesis the ovary is round but that length growth proceeds faster than width until the fruit attains one-third its full length with the result that the mature fruit is oval (fig. 2, *I*). The

inference is that the differential growth rate which Houghtaling (*5*) found to cease at anthesis continues much longer in this case. Histological studies showed that cell division does continue thus after anthesis. This is more evidence that large-oval is not the result of the *o* gene.

THE DEVELOPMENT OF PEAR SHAPE

Pear shape has been something of a puzzle since the earliest genetical work with tomato. It was first used as the designation for lengthened fruit shape but was later replaced by "oval." While an examination of developing ovaries reveals that oval shape may be detected long before anthesis, pear shape is often not evident until near blooming time. At anthesis in pear-shaped varieties, the ovaries have the appearance of being molded into pear form by the constricting effect of the cone forming corolla tube around the pistil. Longitudinal sections at various times show that the seed cavities extend internally into the base of the ovary until the external, necked appearance is visible under a hand lens (fig. 2–*A*, *B*, *C*).

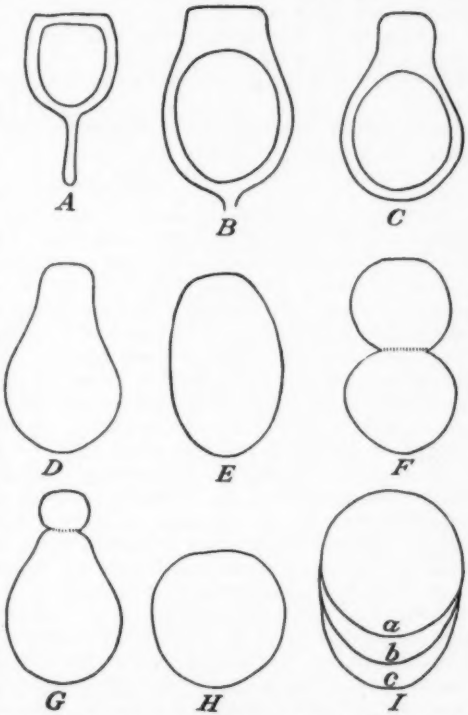

FIGURE 2.—Development of pear shape: *A*, Some time before anthesis; *B*, at anthesis. *C*, Mature fruit; note shift in location of locule. *D–G*, Fruit shape variations to be found on the same plant. *F* and *G* undoubtedly having been caused by persisting corolla tube constriction. *H*, Fruit resulting from a blossom of the pear variety from which the corolla tube was removed some time before anthesis. *I*, Shape changes in large oval tomatoes such as Ohio Red: *a*, At anthesis; *b*, blossom faded; *c*, from one-third mature fruit length to maturity.

Genetically identical stocks raised in different environments may vary in the degree of pear shape in mature fruits. For example, two groups of plants of a Red Pear variety were raised, one in the field and the other in the greenhouse. The crop in the field was typically pear-shaped, the one inside had few distinctly pear-shaped fruits. Even on the same plant some specimens may be markedly pear-shaped and others oval but not pear-shaped. Occasionally fruits may be grooved in the middle or with the neck of the fruit showing an added constriction. When these were first seen on plants in commercial breeding blocks the plants were discarded as being rogues, but, an examination disclosed remnants of the corolla tube. This suggested that pear shape might be induced by the fused corolla tube. If this is so, an early removal of the corolla should result in the production of fruits without a neck. Such an operation on blossoms of the pear-shaped tomato did produce this effect in some cases as will be noted in

figure 2, *H*. Pear shape may therefore be a secondary effect of a constricting corolla. This does not mean that this tendency is not inherited, but it does throw some light on the reason its inheritance is difficult to study. Not only was pear shape not evident in some fruits developing from flowers the corolla tubes of which were removed early, but in several instances, such as the one shown in figure 2, *H*, the resulting fruit was round rather than oval, suggesting that oval shape itself may be an induced condition.

Typical pear-shaped fruits were also produced artificially by constriction during the period of cell division by the use of a thread tied around the blossom buds of ordinary plum-shaped tomatoes at an early stage and removed at anthesis.

DISCUSSION

Any study of the inheritance of fruit size and shape in the tomato plant reveals that the matter is complex. Environment is well known to affect both characters. The vegetative vigor of a plant may greatly affect the size of a fruit. This being true, genes not directly related to size and shape of the fruit in such a plant as the tomato but which affect its general vigor and other plant characteristics, as suggested by MacArthur (*12*), have their effect. When genetically different plants are grown where there is variation in external conditions, such as temperature, light, soil, and parasites, the effect of the different genes will be modified. Even on the same plant, conditions differ enough in different parts to affect size and shape.

During the 6 years of this investigation many tomato crosses were made and studied in an attempt to learn more of the development and inheritance of fruit shape and size. It was found that there is a high degree of association between locule number, size, and shape, and that an association exists between qualitative first chromosome factors and locule number. By means of partial correlations the existing correlations between shape and weight were shown to be due at least in part to their mutual correlation with locule number. Locule number was found to segregate in a ratio of 3:1 of few to many-loculed plants in F_2 populations, and in a ratio of 1:1 in backcrosses, the dominant group carrying two to three locules, while the recessive group carries three and one-half and more locules. A round tomato with many locules has been shown to be *oo lclc* by means of crosses with two-loculed round tomatoes and two-loculed oval tomatoes. Linkages with first chromosome genes were calculated which demonstrate that the *Lclc* locus is on the opposite end of the chromosome from *Dd* beyond the locus of *Ss*. The presence of this gene for locule number may have an effect on size of fruit by an increase or decrease in the number of locules without affecting size of locule. This relationship is not fully demonstrated and even if it were it would not account for all size differences. Obviously there are other size genes, since oval two-loculed tomato varieties are known which differ from each other by several hundred percent. The same is true of round two-loculed sorts. More work is needed on the locule number and size relationship.

The existence of the genes *Lclc* is of great importance in accounting for variations in tomato shape. The genes *Oo* which affect gross fruit shape through their effect on locule shape together with *Lclc* which affect shape by varying the locule number, provide a key for explaining gradations as follows: *OLc*—round, *Olc*—oblate, *oLc*—oval, and *olc*—round.

Correlations between the ovary and the fruit have confirmed the findings of others that with most varieties the size and shape of tomatoes are rendered predictable at blossoming time by measuring the ovaries. Observations have shown marked effects of environment on pear shape. Mature fruits have been observed in which a persistent corolla tube has obviously caused constrictions. Early removal of the corolla has resulted in fruits without pear shape on plants where control blossoms produced pear shape. These suggest that pear shape is probably induced by the constricting effect of the fused corolla tube.

The importance of such developmental studies is emphasized by the differentiation of two groups of oval tomatoes recorded in this paper. The one type symbolized by *o* is composed of the usual comparatively small-fruited varieties in which the final shape and size is fixed at blossoming. The other, including the larger-fruited varieties, may due to a complex that causes continued elongation. When this is superimposed on the genetic complex producing a round tomato, it becomes oval, and upon an oval tomato, extreme length is the result; but when added to an otherwise oblate tomato the result is round. The nature of this complex and its inheritance has not been cleared up. In order to clarify this it would seem desirable to use only *LcLc* varieties for both parents. This would require either the discovery of, or the synthesis of, a pure elongated *LcLc* stock which might require considerable time and labor. If this were done it is possible that the existence of a single major gene for continued elongation could be demonstrated. Such confusion as has been observed to date may be only the effect of *Oo*, *Lclc*, and perhaps other recognized genes such as *Ff*.

Practical breeding work has demonstrated the possibility of recombining *Oo*, *Lclc*, and the factors for elongation. Comparatively large, very oval, few-loculed strains from Iowa State College, when crossed with Early Jumbo, a large oblate, have resulted in lines closely resembling Oxheart. Oxheart, a large, many-loculed heart-shaped fruit, when crossed with Yellow Cherry, resulted in segregates that were very oblate, small, and many-loculed. Ohio red crossed with Yellow Pear has given extremely long, few-loculed segregates. While these examples are not offered as a definite proof of the existence of a single gene for added elongation they do point toward no more than a rather simple complex.

CONCLUSIONS

1. There is significant correlation between shape, size, and locule number in tomato fruits. Part of the correlation between shape and size is due to their mutual correlation with locule number.

2. A major gene for locule number exists. Two to three locules, *Lc*, is dominant over three and one-half or more locules, *lc*.

3. The locus of *Lclc* is on the first chromosome beyond *Ss* with about 20 percent cross-over.

4. The effect of *lc* when associated with *o* is to produce a phenotypically round tomato.

5. With most of the tomato varieties, the size and shape of the matured fruit is predictable from ovary measurements at anthesis.

6. Pear shape is probably due to the constricting effect of the fused corolla tube.

7. Oval tomatoes in some cases are the result of genes other than *o*, the effect of which is to continue the elongation of the ovary after anthesis.

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AN UNDESCRIBED POTATO DISEASE IN WEST VIRGINIA[1]

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HISTORY

In 1931 a disease was observed on a few scattered plants of potato (*Solanum tuberosum* L.) in Preston County, W. Va. Since then it has spread steadily and has become a limiting factor in potato production in certain districts of the State. This disease has been observed also in Pennsylvania and Maryland. Altitudes, soil types, and moisture conditions have not appeared to be significant factors in the distribution or the intensity of this disease, nor does it appear to be influenced by fertilizer treatments.

All strains of Rural, both russet and smooth, are very susceptible. The Irish Cobbler strains and Green Mountain, Early Rose, Chippewa, Katahdin, and other varieties grown in West Virginia have also been attacked. The early varieties are less frequently diseased, possibly because they mature before the peak of infection is reached.

The origin of the disease is unknown. Some observers believe that it has been present for 12 years or more. The importance of the disease with respect to yield depends largely upon the stage of development of the potato plant at the time of infection. One field which showed nearly 100 percent severely infected plants in 1935 yielded less than 10 bushels of United States No. 1 tubers per acre. Similar cases were recorded in 1936.

DIAGNOSIS

MACROSCOPIC SYMPTOMS

The first external symptoms are a dwarfing of the upper stem, accompanied by a folding upward and narrowing of the terminal leaflets (pl. 1, *A*). This is soon followed by a paling of the foliage, and in Rural varieties by the appearance of a purplish tinge on the margins of the younger leaflets. Soon the rest of the foliage fades, and in the Rural varieties the stem assumes a purplish color. Wilting follows rapidly and the plants make an attempt to recover by sending out axillary shoots which may reach a length of 3 to 6 inches. These shoots present symptoms similar to those observed on the primary terminals. They are short-lived, and the whole plant dies a week or 10 days later. Aerial tubers have been observed rather frequently but may be the result of other causes.

The roots have a dull color, are reduced in size and length, and break easily when the plants are pulled. Tuber production is dependent upon the time of infection. Plants which become infected before tuberization produce few if any tubers of marketable size. Only the plants which become infected at about the time of maturity may pro-

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duce a normal yield. The intermediate periods of infection induce corresponding effects upon yield.

While the time of infection and the period of incubation have not been determined, it is known that the plants generally die within 2 weeks after the appearance of the first unmistakable symptoms.

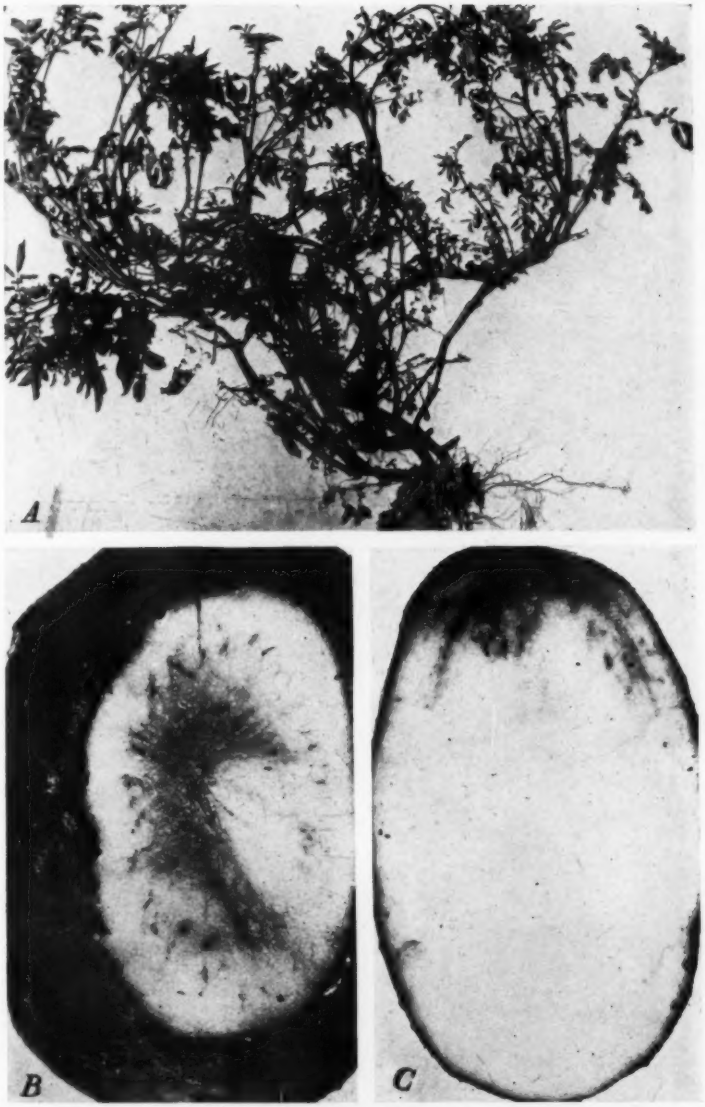
The internal macroscopic symptoms which are of the greatest diagnostic value are as follows: A general brownish discoloration of the vascular region which begins at the base of the stem prior to the appearance of foliage symptoms, and progresses through the roots, stem, and stolons into the tubers, and an occasional rusty spot which is usually present in the lower pith region of the stem and often throughout the stem pith. Only the cortical region shows necrosis in cross or in longitudinal sections of the roots. The stolons present a picture similar to that of the stem. Necrosis in the tubers is very characteristic. There is a pronounced and intense brown discoloration at the point of rhizome attachment. From this region inward a branched system of necrotic woody strands extends through the vascular region, involving one-quarter to one-third of the entire vascular system. At the same time numerous strands, often dendritic in appearance, extend less deeply into the internal storage parenchyma. A transverse section of the tuber just inside the point of stolon attachment discloses a picture similar to that found in net necrosis, although it is less extensive in the internal parenchyma (pl. 1, *B*, *C*). The tubers do not rot in storage unless invaded by secondary organisms.

MICROSCOPIC STUDIES

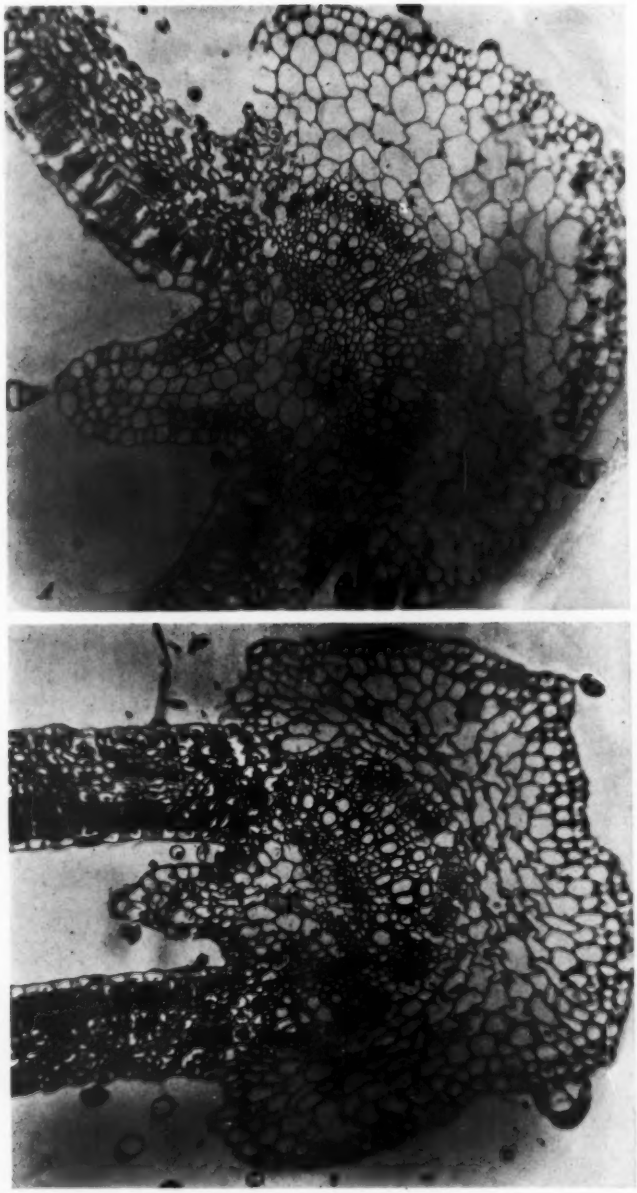
The material for study in 1935 was obtained from a commercial field of Russet Rural in Preston County. This field was relatively free from all other diseases. The material was fixed in formol-acetic-alcohol, and the usual methods of dehydrating and embedding in paraffin were used. Stem sections were cut 15 microns and all other parts 10 microns in thickness. Flemming's modified triple stain was used exclusively. The material for study in 1936 was obtained from the descendants of the foregoing crop of 1935. In addition to this, specimens from caged and uncaged healthy plants were obtained for comparison. All sections were cut 10 microns in thickness.

The most conspicuous anatomical feature to be noted in the young leaflets is the marked changes which take place in the palisade and mesophyll tissues (pl. 2, *A* and *B*). While the palisade cells in the diseased leaflets do not reach normal size, the most remarkable change is brought about by the laterally crowded condition of these cells and by the consequent closing of the large intercellular spaces which are so evident in healthy palisades. A secondary palisade is frequently found in the diseased leaves. Similar changes take place in the spongy mesophyll, where the intercellular spaces are greatly reduced by the crowding of the cells. The effect of these changes is reflected in the narrowing of the lamina, but no marked reduction in thickness has been seen. There is no appreciable change in the tissues of the petiole if necrosis is not present.

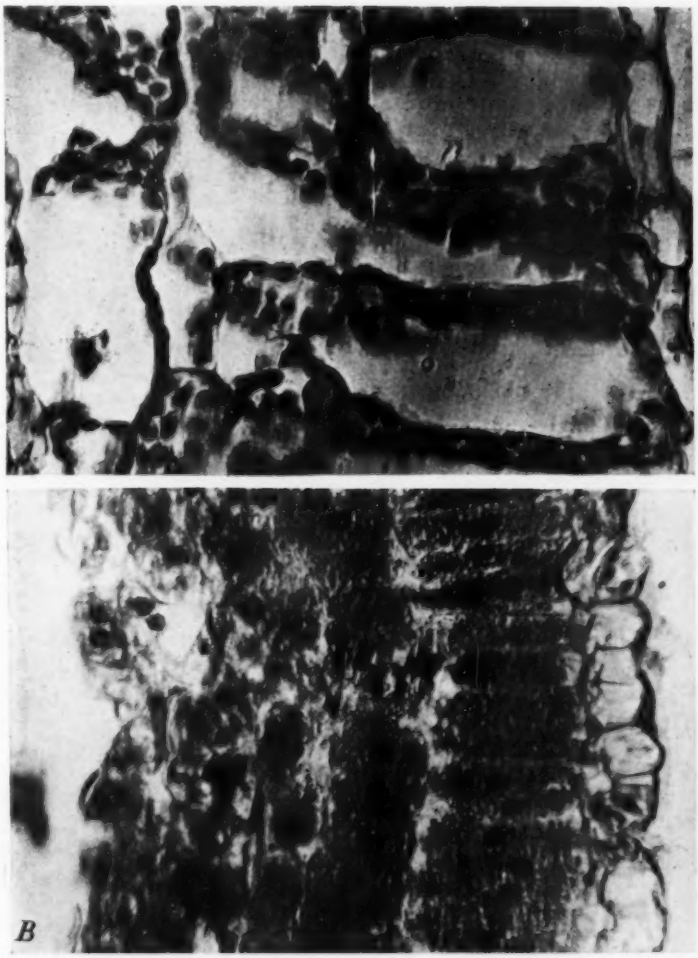
These changes are accompanied by a depletion of the chloroplasts during which they lose their starch as well as their normal vacuolated appearance. Consequently they appear empty or contain only a few stainable threads and soon they lose their regular outline, collapse,



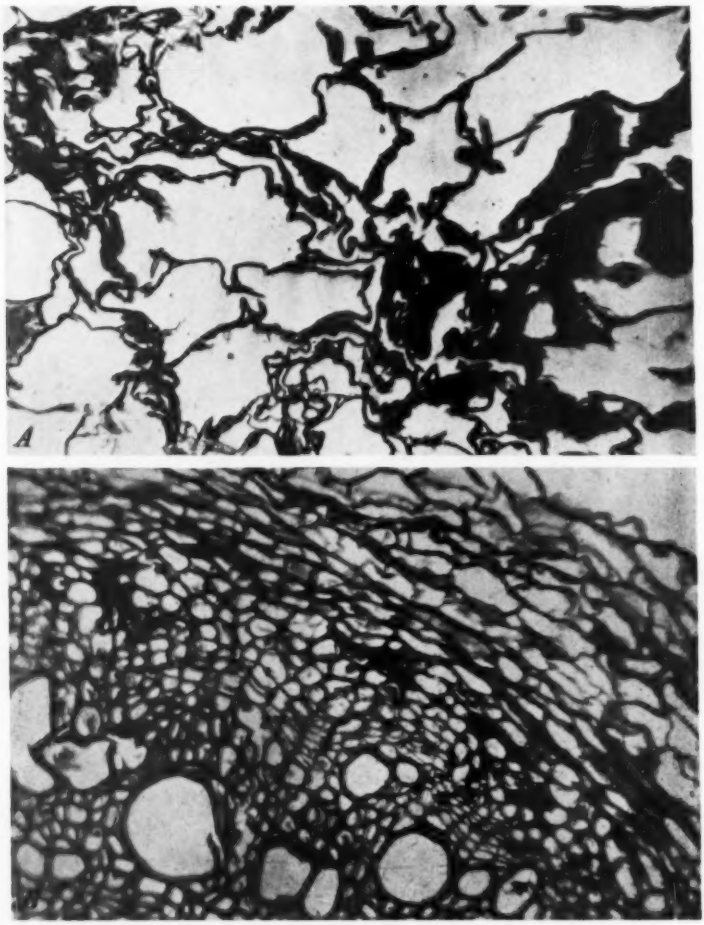
A, Late symptoms of a diseased Russet Rural potato plant showing upward folding of terminal leaflets and development of axillary shoots. *B*, Transverse section of stem end of tuber showing necrosis. *C*, Longitudinal section of tuber showing necrosis in vascular region and in the fundamental tissues.



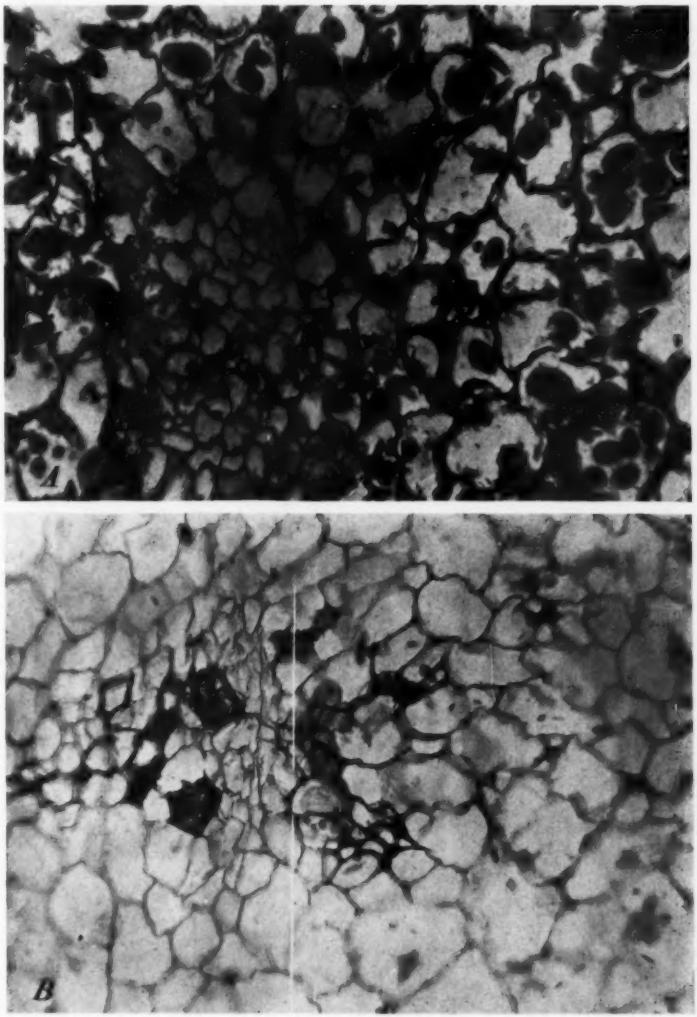
A, Transverse section of healthy petiole and laminae showing intercellular spaces in palisade tissue; note the angle of attachment of the laminae to petiole. × 130. *B*, Transverse section of diseased petiole and laminae showing absence of intercellular spaces in palisade; prominent nuclei throughout palisade; and the sharp angle of attachment of laminae to petiole. × 130.



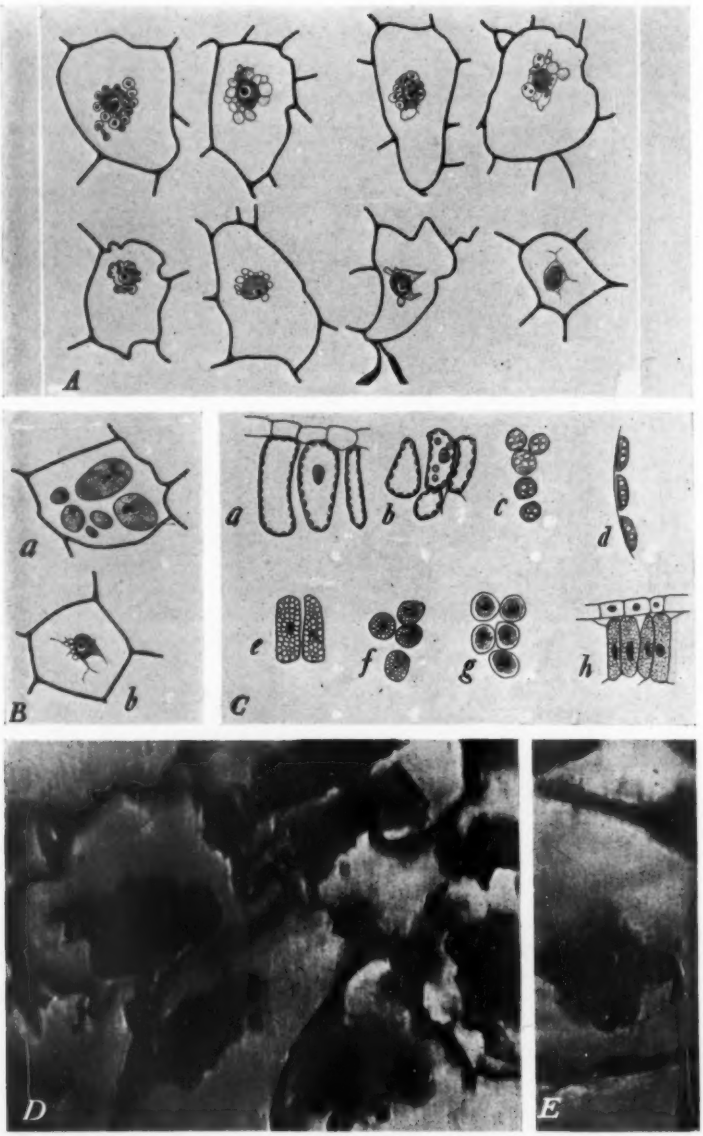
A, Healthy lamina; note large intercellular spaces and normal shape and position of chloroplasts. × 750. *B*, Diseased lamina showing absence of intercellular spaces; prominent nuclei; secondary palisade; and depleted chloroplasts which are diffused throughout the cells. × 750.



A, Transverse section of pith necrosis in lower stem with collapsed cells and granular deposit. × 130.
B, Transverse section of root showing phloem and cortical necrosis. × 240.



A, Transverse section through phloem of healthy tuber showing normal storage of starch grains. × 250.
B, Transverse section of diseased tuber showing phloem, xylem, and parenchyma necrosis with vessel filled with granular mass; note absence of starch grains adjoining necrotic areas. × 250.



A, Progressive stages in starch dissolution. The black center of small spheres is the hilum which disappears in late stages of dissolution. *B*, *b*, Final stage of starch dissolution leaving a threadlike mass around nucleus; *a*, healthy cell with normal starch grains. × 275. *C*, *a*, Normal palisade cells, × 275; *b*, normal mesophyll cells, × 275; *c*, normal chloroplast in mesophyll, × 980; *d*, normal chloroplasts in palisade, × 980; *e* and *f*, palisade and mesophyll cells, intermediate stages of depletion of chloroplasts. Vacuoles have disappeared leaving chloroplast membranes (in palisade and mesophyll cells) × 375; *g*, mesophyll cells, late stage of chloroplast depletion with remains grouped around nuclei, × 375; *h*, palisade cells, late stage of chloroplast depletion with remains scattered throughout palisade cells: nuclei prominent, × 375. *D*, Intermediate stage of starch dissolution showing starch grains aggregated around nuclei. × 980. *E*, Late stage of starch dissolution showing a group of spheres around nucleus. × 980.

leave their parietal position, and become scattered throughout the cell (pl. 3, *A*, *B*). In the mesophyll cells the depleted chloroplasts have a marked tendency to cluster about the nucleus (pl. 6, *C*, *g*). In later stages of disorganization they stain more deeply than the normal chloroplasts.

At this stage of disorganization the nucleus undergoes a marked change. The nuclear membrane takes a much darker stain, appearing nearly black and the chromatin loses its affinity for the gentian and becomes masked with safranin so that the nucleolus is differentiated only as a denser spherical body. The hyaline zone which is conspicuous about the nucleolus under normal conditions becomes masked by the dense safranin stain (pl. 6, *C*, *e*, *f*, *g*, *h*). The darker staining nuclei become more prominent and form a dark line which is conspicuous throughout the palisade (pl. 2, *B*).

Phloem necrosis is one of the early microscopic symptoms in the stem and appears first in the basal region. The necrosis may be confined to one phloem group, either internal or external, but as the disease progresses it appears in other phloem groups where the sieve tubes and companion cells are most generally involved. The necrosis also extends into the adjacent fundamental tissue. With the triple stain these necrotic areas become sharply marked through the intense safranin stain taken up by the walls of the cells involved. As soon as the phloem necrosis is well developed, a granular mass appears which may fill the dead cells and in some cases also a large part of the lysigenous space which frequently develops in the necrotic area. The mass is made up of granules of various sizes and shapes so densely crowded that their structure is obscure (pl. 5, *B*). In unstained sections this mass is yellow; in sections stained by the triple method, the mass stains heavily with safranin. The necrosis of both the internal and external phloem tissues develops in a discontinuous manner throughout the stem, the secondary shoots, the stolons, the stem end of the tuber, and the protophloem of the roots, where it becomes very extensive. This discontinuous development of the phloem necrosis is readily demonstrated in longitudinal sections, where the necrotic areas show a considerable variation in extent.

Examination of stained transverse sections of the xylem from diseased plants does not show the marked effect which would be expected from the extensive browning of the tissues. Most of the xylem takes the normal safranin stain except for an occasional vessel or small group of vessels lying near the cambium, the walls of which take a darker safranin. Such cells invariably contain a finely granular mass quite different in appearance from the masses found in the phloem. Those in the xylem are more spongy, frequently cytoplasmic in appearance, and take a bright gentian stain, in contrast to the safranin affinity shown by the masses associated with necrosis in the phloem. In some cases these inclusions in the vessels are so dense as to appear to occlude the cell; in other cases they may be of a very loose spongy formation. An examination of these cells in longitudinal section shows that these tracheal inclusions rarely, if ever, completely occlude the vessel. They extend only a short distance, usually not more than double the diameter of the cell. In unstained sections the masses are brown. They have been observed in the xylem of the main stem, petioles, stolons, and tubers (pl. 5, *B*). Such inclusions have not been

seen in the xylem of the secondary shoots and roots, but this may be due to insufficient search in these organs.

The rusty spots which are commonly associated with the basal region of the stem pith are not as prominent microscopically as they are macroscopically. In the early stages single cells are found scattered throughout the parenchyma, the walls of which take a deep safranin rather than the normal orange stain. In later stages a number of such cells may be seen arranged in an irregular group which contains one or more cells with granular or globular masses similar to those found in the necrotic phloem and showing the same stain reactions. These cells eventually collapse to form a tangled mass (pl. 4, *A*). Necrosis of the internal parenchyma is also present in the tuber but is not evident from macroscopic examination. In the cortical region of the stem, stolons, tuber, and root the effect is like that described for the internal parenchyma of the stem.

One of the most striking effects of the disease is found in the storage cells of the tuber which are normally filled with large starch grains (pl. 6, *B*, *a*). Prior to the first appearance of necrosis the starch grains migrate to the nucleus and completely surround it. Here they undergo dissolution. During this process the starch grains gradually decrease in size, lose their oval shape, and tend to become spherical. The hilum, when present, appears to become more prominent because it remains unchanged while the starch grains decrease in size. Later the hilum disappears and all that can be seen of the outline of the starch grains are the small empty spheres which remain closely surrounding the nucleus (pl. 6, *A*, *B*, *b*, *D*, *E*). Finally these fuse together and form a stringy, often granular mass.

The nucleus shows no pronounced change in its staining reaction or appearance prior to the final dissolution of the starch spheres. At this time the contents of the nucleus lose their affinity for the gentian violet and stain dark with safranin. In general, its appearance is the same as that described for the nuclei in the palisade cells during the disorganization of the chloroplasts. In some cases the shape of the nuclei of these storage cells becomes irregular. This phenomenon of starch dissolution, followed by necrosis, continues progressively in all storage cells of the plant as long as the stem remains alive.

SUMMARY

A potato disease, the origin and etiology of which are unknown, has been under observation since 1931 in West Virginia. This disease has become a limiting factor in potato production in some areas.

The first external symptoms are characterized by a dwarfing, paling, and upward folding of the terminal leaflets. Within 7 to 10 days the vines wilt and die. The vascular region of the stems, tubers, stolons, roots, and numerous regions in the pith of the stem turn brown. A discontinuous dendritic necrosis of the stem end of the tuber is one of the principal characteristics of this disease.

The anatomical features of the young terminal leaflets consist of a lateral crowding together of the palisade and mesophyll cells, failure to reach normal size, and the elimination of the intercellular spaces. These changes are accompanied by a depletion of the chloroplasts and the loss of their starch grains and vacuoles. Finally, the chloroplast

membranes disintegrate and diffuse throughout the cell. The nuclei retain their normal shape but stain heavily with safranin.

An extensive necrosis exists in the phloem and adjacent parenchyma, and to a lesser extent in the xylem and fundamental tissue. A granular deposit occurs in the necrotic areas which sometimes show lysigenous cavities. The vessels are sometimes filled with a granular material differing in staining reactions from that in the phloem and parenchyma.

Before necrosis takes place the starch grains migrate toward the nuclei, become spherical, undergo a gradual dissolution, eventually leaving behind hyaline spheres which fuse together to form a thread-like mass. The nuclei of the storage cells generally retain their normal shape and take a darker stain.

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